

## 20.

## Chemical Sensory Reactions in the Mexican Blind Characins.

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(Plates I-III; Text-figures 1-9).

## INTRODUCTION.

In the attempt to understand more fully the various features of the behavior and possible evolution in progressive stages of the changes in the Mexican blind fish of the *Astyanax mexicanus* (Philippi)—*Anoptichthys jordani* Hubbs and Innes series it is, of course, necessary to obtain some evaluation of the various sensory systems. The present contribution is therefore such an attempt to analyze the differences, if any such be present, in the chemical sensory equipment of these fishes. Both the structural and behavioristic elements involved are herewith discussed.

Recently a new form has been discovered in another cave by one of Dr. Hubb's collectors, as noted by Tafall (1942 and 1943) and Breder (1943). This form is evidently still further advanced in eye and pigmentation loss. We have been fortunate in obtaining some of these alive through the good offices of Mr. B. Dontzin who visited this cave, Cueva de los Sabinos, in 1942 for that express purpose. Tafall (1943) has made an important contribution to the ecology of both La Cueva Chica and Cueva de los Sabinos. In his study of conditions he enumerates the species of aquatic organisms and discusses at length the fishes of these caves, making comparisons with the fauna of the cenotes of Yucatan. The taxonomic considerations involved are under study by Dr. Hubbs, while the present paper discusses some of the anatomical and behavioristic items of this form compared with fishes from La Cueva Chica and normal-eyed river fishes. The details of the ocular anatomy and reactions to light in this form are discussed by Breder and Gresser (Ms.) A popular version of the history of these studies may be found in Bridges (1940 and 1943).

As in all studies concerned with the chemical senses of fishes it was found much more difficult to obtain definitive results than in the earlier studies on reactions to light of Breder and Gresser (194a and b). We feel, however, that enough data have been accumulated to indicate the basic nature of these features in reference to the behavior of the forms involved and the apparent nature of the evolutionary changes in progress.

The work was carried on in the Department of Animal Behavior of the American Museum of Natural History. We are grateful to Miss Annette Bacon for advice in connection with the mathematical treatment and for editorial assistance and to Lt. James W. Atz for numerous helpful comments.

## THE NASAL CAPSULE.

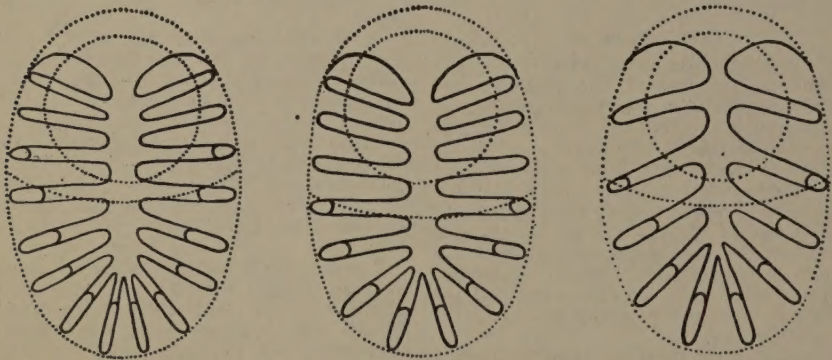
Serial sections and gross dissections of the nasal capsule of each of three types under comparison showed that these were rather typical characin structures, but that each form showed a slight but significant morphological difference, in which La Cueva Chica material was intermediate between the eyed river fish from the Rio Tampaon and Cueva de los Sabinos specimens. These differences were chiefly in the reduction of the number of sensitive lamellae and a decrease in the depth of the pit, proceeding from the river to the Cueva de los Sabinos stocks. The functional significance of the sense of smell involved in these changes is not entirely clear on an anatomical basis. The funnel-like flap that serves to direct water into the anterior opening increases in size slightly in this series as is indicated in Plate I, wherein the right hand flaps are shown in profile.

No histological difference could be found in the olfactory epithelium from one form to another. Text-figure 1 shows a conventionalized reconstruction of the ridges in relation to the positions of the anterior and

\* The studies embodied in this paper were started as part of the work in the graduate course in ichthyology given by the senior author at New York University. Subsequently the studies were expanded to their present form.

posterior nares of each type. As is indicated, in the river fish there are eight pairs of ridges, in La Cueva Chica material seven and in the Cueva de los Sabinos only five. A selected, approximately homologous section of each of these is shown photographically in Plate II. Here the raising of the floor of the pit and the more fully exposed positions of the finger-like lamellae sections in the Cueva de los Sabinos fish is indicated. It is also evident from this plate that in the river fish some of these lamellae become anchored at their distal ends more anteriorly and are more numerous than in either cave fish. The nasal openings of the Cueva de los Sabinos fish are larger and offer more direct exposure of the ridges to the exterior.

*vulgaris* Cuvier to number about ten pairs, figures the nasal pits of *Gadus morrhua* Linnaeus and *Mugil chelo* Linnaeus and discusses nasal anatomy in general. Adrian and Ludwig (1938) indicate about 31 pairs for *Ameiurus nebulosus*. Busnita (1932) discusses the histological details of the sensitive epithelium of *Carassius*, while Laibach (1937) shows that in development the nasal lamellae increase in number from four to thirty-six pairs as *Anguilla vulgaris* Linnaeus transforms from the leptocephalus stage and grows to adult size. That possible size or age difference could not have anything to do with the differential counts in the characins has been checked. Specimens long before reaching the size of a small adult



TEXT-FIG. 1. Diagrammatic representation of the sensory ridges in the nasal capsules of Mexican characins. Left: from Rio Tampaon. Middle: from La Cueva Chica. Right: from Cueva de los Sabinos. Based on reconstruction from serial sections and gross dissections. In each case the left nostril is represented in plan view with the fish facing upward. The dotted outlines represent the anterior and posterior external openings. See also Plates I and II.

Whether these features are to be interpreted as a reduction of nasal acuity, on the basis of a reduction of sensitive areas, or an increase, based on the greater exposure of the remaining areas and a larger funneling flap, is something that must wait on the availability of a larger stock of the Cueva de los Sabinos fish suitable for a more extended experimental analysis.

There is considerable variation in the nasal anatomy of teleosts as is well indicated by Liermann (1933) who figures the lamellar construction of *Carassius carassius* Nordmann, *Anguilla vulgaris* Flemming, *Perca fluviatilis* Linnaeus, *Pleuronectes flesus* Linnaeus, *Anmodytes tobianus* Linnaeus, *Zoarces viviparus* Linnaeus, *Gasterosteus aculeatus* Linnaeus and *Syphnostonum typhle* Linnaeus. Tretjakoff (1930) shows the lamellae of *Scomber scombrus* Linnaeus to be practically radially arranged and the platelets to number about twenty. Matthes (1934) shows the lamellae of *Tinca*

show a fixed and constant number. If such changes take place with the development of these characins it must be at a size below which we have comparative material. As would be expected the nasal capsules of the cyprinids more closely resemble those under discussion than do the other species more remotely related.

Many other authors have described the nasal anatomy of a variety of fishes including figures of *Zeus faber* Linnaeus and *Gadus morrhua* by Berghe (1929), *Prototterus* by Fullarton (1933) and various Heterosomata by Chabanaud (1927 and 1936). Derscheid (1924) discusses at length the nasal capsules of the Isospondyli with figures of the anatomy of no less than thirty species well distributed throughout the order. Most of these have numbers of lamellae much higher than our characins but the following approach them in this sense: *Chauliodus sloani* Bloch and Schneider with five, *Pantodon bucholtzi* Peters with ten



pairs, *Heterotis niloticus* Cuvier and Valenciennes with sixteen radially arranged, while seven species of Mormyridae of the genera *Marcusenius*, *Mormyrus*, *Genyomys* and *Mormyrops* range from six to eight pairs of lamellae. In general terms these Isospondyle nasal pits resemble those of the Ostariophysi about as closely as these two orders resemble each other in most respects. Frisch (1941a) indicates ten pairs of lamellae in the nasal pit of *Phoxinus laevis* Agassiz. It is evident from this brief survey of the literature, moreover, that the sensitive lamellae of these characins approach the lower limits in number much closer than they do the upper. From this and general considerations we cannot but conclude that the fishes in question are not nearly as well provided for in regard to olfaction as are many other species, including a number with especially well developed eyes.

#### THE TASTE BUDS.

Complete serial sections of one of each of the three forms has made possible the reconstruction of the distribution of the taste bud tracts on the exterior surfaces of the fishes. The distribution of these in each is indicated in Text-figure 2. As each sectioned fish varied slightly in bodily proportions, all have been reduced to a standard outline in order to facilitate comparison. The actual outline of each fish is indicated by the small inserts. The dots on the surface of the outline are indicative of the positions of gustatory areas and are not intended to represent individual taste buds in terms of absolute number. It is clear from this figure that there is a marked increase in the areas of gustation from the river fish to those from Cueva de los Sabinos. This is the first unequivocal positive change that has been found in this series. Other changes found, excepting perhaps only the nasal pits, have all been in the nature of reductions, Breder and Gresser (1941a) and Breder (1943). It is to be noted in this figure that the optic area in La Cueva Chica stock, 7th generation of aquarium-reared fishes, is scarcely encroached upon by taste buds whereas in the more advanced Cueva de los Sabinos material this area is well covered by them. The former had a sunken eye, uncovered, lacking a lens and similar to the fish of Plate II of Breder and Gresser (1941a). While the general form of the chief tracts of taste buds is fairly constant it is to be noted that with the increasing number there is evidently some slight translocation of some of the areas of taste. Also, along with this increase in taste areas, chiefly on the head, there is evidently some slight reduction of them on the caudal fin, but this is perhaps little more than a matter of individual variation. These findings have been

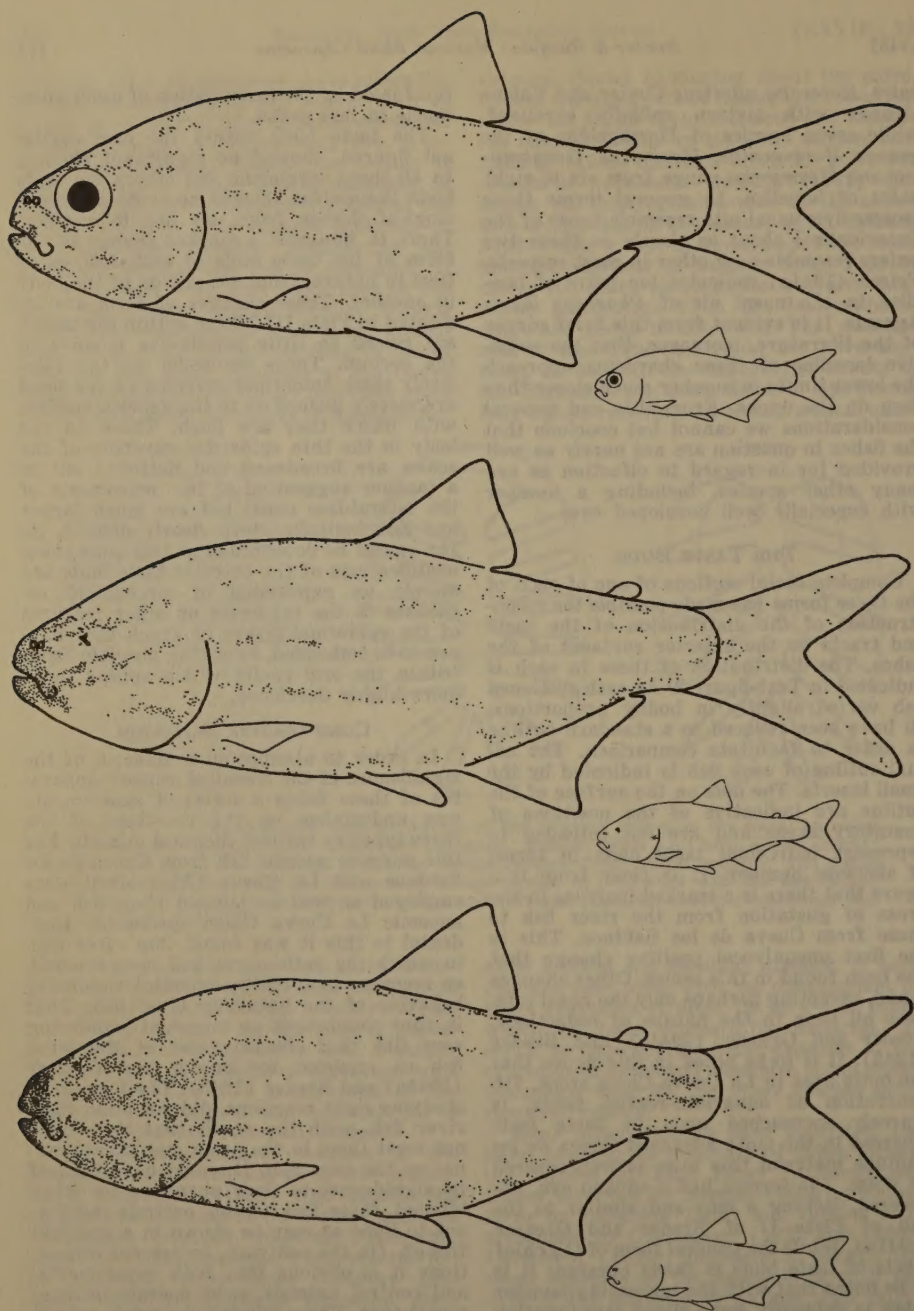
reinforced by the examination of other specimens in less detail.

The taste buds within the oral cavity, not figured, showed no significant change. In all three specimens and others the taste buds themselves showed no evident morphological change from one fish to another. There is, however, a marked change in the form of the taste buds of each fish, identical in nature, from one region of the body to another. These changes of form are indicated in Plate III. Those within the mouth are raised on little papilla-like mounds in the corium. Those embedded in the relatively thick epidermal covering of the head are merely pushed up to the general surface with which they are flush. Those on the body in the thin epidermal covering of the scales are broadened and flattened out in a manner suggestive of the neuromasts of the lateral-line canal but are much larger and histologically more clearly defined. So far as can be determined at this time these modifications of the exterior taste buds are merely an expression of mechanical responses to the thickness or other features of the epidermal layers in which they happen to be imbedded, excepting possibly those within the oral cavity which appear to be more highly developed.

#### COMPARATIVE BEHAVIOR.

In order to attempt an evaluation of the significance of the chemical sensory apparatus of these fishes a series of experiments was undertaken on the reactions of the three types to various chemical stimuli. For this purpose normal fish from Cueva de los Sabinos and La Cueva Chica stock were employed as well as blinded river fish and anosmic La Cueva Chica specimens. Incidental to this it was found that river fish, in which the optic nerve had been severed, on recovery took on the essential wandering behavior of the naturally blind fish. That is, they commenced an incessant wandering very like that characteristic of the blind fish as reported by Breder and Gresser (1941a) and Breder (1943). The former, in studying light reactions, did not blind their river fish, with the result that they could not treat them in the same statistical manner as the cave fish. It would have been of no significance to their purposes, for when blinded, these fish become entirely indifferent to light as may be shown in a gradient trough. On the contrary, in present connections it is obvious that both experimental and control animals must operate without visual cues. These blinded river fish quickly became accommodated to their new status and lived on as well as the cave fish and found their food in an identical manner.

The anosmic fish were produced by means of electro-cautery of the nasal capsules. On recovery these showed little basic differ-



TEXT-FIG. 2. Distribution of exterior taste buds on Mexican characins. Upper: from Rio Tapaon, 34 mm. in standard length. Middle: from La Cueva Chica, 29 mm. in standard length. Lower: from Cueva de los Sabinos, 32 mm. in standard length. Reconstructed from serial sections. The small inserts represent the true outline of the fish in each case. The large outlines of identical nature are based on a typical form to which the taste bud distribution has been referred in order to facilitate comparison. See text for full explanation.



ence from the normal fish except that they did not find their food as quickly.

Beigel-Klaften (1913) discussed the regeneration of the nasal organ in *Tinca vulgaris* and in *Cyprinus carpio* Linnaeus. With the electro-cautery method employed in the present experiments, examination as well as the subsequent behavior which remained the same after several months showed there was clearly no reconstruction of the apparatus or ability to smell.

Much of the work on comparative behavior was undertaken in a trough 22 inches wide by 39 inches long with water to a depth of two inches. This was optically divided into two like compartments by means of a string drawn taut above the surface of the water so that there were, in effect, two parts, each 18½ inches by 22 inches. Burettes were suspended, one over the end of each, five inches from the end of the trough along its mid-line, so that the two sources of chemicals were 29 inches apart. In these troughs the positions of the fishes, in reference to the two compartments, were checked at five second intervals in a manner similar to that used by Breder and Gresser (1941a and b) for their studies on light reactions. In most of the experiments four fish were used at a time as more than that number were generally too difficult to keep accurate check on simultaneously. The end at which the chemicals were administered was alternated with each experiment in order to overcome any possible inherent bias in the trough.

For studies involving the circling movements of normal and semi-anosmic fishes another trough of 39 inches by 17 inches was employed, but which was otherwise similar.

After each experiment, the trough was flushed and tempered water was substituted for the contaminated water of the last test. The temperature of the water was maintained between 21° and 25° C. throughout the experiments described.

#### Chemical Repellents.

In the early exploratory part of the experiments it was found that acetic acid operated well as a repellent for these fishes and as a consequence it was largely used in these studies. Citric acid was also used but was found to show no advantage. Ammonium carbonate was also employed. The simplest and most satisfactory method of application tried was to allow dilute solutions to drip from a burette at as nearly a uniform rate as possible at one end of the trough while plain water from the trough dripped at a similar rate at the opposite end. Any possible, but not readily detectable, mechanical disturbance due to the slow inflow was thus compensated. The basic data of these experiments are given in

Tables I, III and IV together with pertinent data on quantities administered and pH values reached.

Table I gives the chemical and related data as well as the positions of the fish in percentage at the repellent end of the trough. Every experiment is designated by a number and letter. Each number indicates the use of a different substance or quantity. The letters indicate separate experiments, of which, in most cases, there were four of one kind. Each period of observation, of which there are eight, indicated at the right of the table, represents 100 observations at 5-second intervals. These values indicate the number of fishes recorded, expressed in percentage. Thus in experiment "1a" under period "1," the value 40 means that during the 100 observations made, 160 fishes were counted out of a possible 400 (4 fishes moving at random under such conditions would show 200 in one compartment or 50% of the total possible). All periods for each experiment, or horizontal row of figures, represents a period of 53 minutes and 20 seconds of observation. The even-numbered periods are consecutive with the preceding odd-numbered periods while an interval approximating a half hour represents the spacing of the beginning of each odd-numbered period. The means of each experiment thus represent an actual observation time of nearly two hours (1 hour, 53 minutes, 20 seconds) with 3,200 observations at five-second intervals.

Table III, together with other data to be discussed later, gives continuations of five of the tests that were carried on for longer periods, the longest reaching 1,900 observations over a period of more than two and one-half hours (2 hours, 37 minutes, 30 seconds), the latter of which were spread over longer units of time as is indicated in the table.

Table IV gives details of periods "5" and "6" of Tables I and II in eight smaller units together with other data. The horizontal rows of figures in this table are all consecutive and represent 200 observations with the means representing 800 observations.

A digest of this data is given in Text-figure 3. Graph A shows the retreat of the fishes during the actual acid-dripping period, based on Table IV, and indicates clearly the similarity in behavior between the blinded river fish, the normal fish from La Cueva Chica and those from Cueva de los Sabinos. It also indicates the difference in behavior of the anosmic fish as compared with the normals and the difference between the reactions of the normals to ammonium carbonate as compared to acetic acid.

Graph B treats the data of Table I in a





TABLE II. EXPERIMENTS WITH ATTRACTANTS.

Each figure concerning the distribution of fish represents 100 observations, the primary means, 400 and the secondary means, 800, except as indicated.

EXP. NO.	FISH USED AND NO.	SUBSTANCE AND % SOL.	CC. USED	PH AT START AND END <sup>2</sup>	PERCENTAGE OF FISH IN SUBSTANCE COMPARTMENT								
					PERIODS OF OBSERVATION								
					PRE-DRIP				SUB. DRIP.		POST-DRIP		
			SUB.	BLANK		1	2	3	4	5	6	7	8
7a	4 blinded river fish	Cane sugar—40%	100	86	7.0	51	53	55	52	53	45	53	62
7b			100	96	7.0	52	46	52	48	60	51	64	62
7c			100	96	7.2	45	45	49	51	51	43	47	34
7d			120	93	7.2	49	53	50	50	55	53	44	51
	Primary means		105	93	7.1	50	49	52	50	55	48	52	52
	Secondary means					49		51		51		52	
8a	4 La Cueva Chica fish (normal)	Cane sugar—20%	70	71	6.8	—	—	51	45	53	50	56	53
8b			90	93	7.0	52	54	46	53	53	52	54	53
8c			93	87	7.0	50	52	54	55	62	53	48	45
8d			100	90	7.0	54	52	52	54	58	56	56	59
	Primary means		88	85	6.9	52	53	51	52	56	53	54	52
	Secondary means					52		51		54		53	
9a	4 La Cueva Chica fish (normal)	Cane sugar—40%	84	99	7.0	60	51	58	69	76	76	73	78
9b			70	56	7.0	50	48	52	52	54	52	53	55
9c			96	62	6.8	47	43	43	45	48	49	42	43
9d			92	68	7.0	50	48	53	55	53	58	54	57
	Primary means		85	71	6.9	52	47	51	55	58	59	55	58
	Secondary means					50		53		58		57	
10a	2 La Cueva Chica fish (anosmic) <sup>1</sup>	Cane sugar—40%	100	96	7.2	51	51	51	49	51	55	53	51
10b			93	69	7.2	50	51	49	49	50	53	56	52
10c			98	79	7.1	50	52	48	49	55	52	61	58
10d			100	86	6.8	50	53	50	57	57	50	53	62
	Primary means		98	82	7.1	50	52	49	52	53	53	56	56
	Secondary means					51		50		53		56	
11a	4 Cueva de los Sabinos fish (normal)	Cane sugar—40%	100	76	7.2	28	45	56	51	65	67	61	53
11b			100	100	7.2	47	37	45	41	40	49	55	49
11c			85	66	7.2	50	49	51	54	60	62	54	50
11d			100	100	7.4	46	49	35	34	35	39	29	31
	Primary means		96	85	7.3	43	45	47	45	50	54	50	46
	Secondary means					44		46		52		48	

<sup>1</sup> Each part of this experiment represents the mean of two experiments, combined here for purposes of this table, as to be comparable with the other experiments.

<sup>2</sup> Cane sugar would not change the pH values but they were checked at the end of each experiment for possible variations due to unknown causes. In all cases the pH values did not vary.

similar manner and serves to indicate the extent of the normal departure from the expected 50 percent. distribution that is found in these fishes by means of the four pre-drip or control periods in each case.

Graph C compresses the data of graph B to double periods and to that extent acts as a smoothing method. In other words, in this graph all the consecutive observations are treated as a unit. This serves to give a more general representation and reduces the incidental details more fully expressed in graph B.

Text-figure 5 show graphically the longer term experiments, the data of which are given in Table III.

It is evident from Tables I and III that some of these experiments ran to the point of lethality. These effects are indicated in

foot notes to the tables. The word "distress" indicates that the fish began moving at higher than normal speeds with their snouts out of water and gave every evidence of being in serious difficulty. In certain cases the mucus became whitish as though coagulated and fell away in small pieces. In all, four fish died either during or immediately after experiments, as is indicated. All the rest survived and at this writing are still living. They gave evidence of rapid and complete recovery in all but the four cases noted.

The significance of these graphs is treated in the discussion.

#### Chemical Attractants.

Experiments with attractants were handled exactly as were those of the chemical repellents. The basic data of these are given





in Tables II, III and IV, and the remarks under the previous head apply equally here. Dissolved prepared dry fish food, the juice of horse meat and cane sugar were used in these experiments. As no statistical difference was suggested in the early work, cane sugar was used for most of the experiments because of ease in standardizing.

A digest of these data is given in Text-figure 4 and is handled in a manner exactly comparable to that of the repellent agents. Text-figure 5 shows graphically the data of Table III.

Table V gives data on the circling movements of normal, anosmic and unilaterally anosmic fishes from La Cueva Chica stock. These experiments were performed in a simple trough. The stimuli, prepared dried food and cane sugar, was given in the solid form by allowing a small quantity to pass down a large glass tube with its end held near the floor of the trough at a time when the fish was at the far end of the trough. In each case a single fish was employed at a time. This was done because it was evident that the excited movements of a feeding fish served to attract the attention of others. This may be a lateral-line effect or could be due to the sound of chewing. The chewing sounds of the pharyngeal teeth of larger species may be heard quite distinctly through a simple "submarine listening tube" made of a stethoscope over the mouth of which is placed an ordinary unperforated nursing nipple.

The essential similarity in the behavior between the normal fish and the fully anosmic in respect to right and left hand turns as compared with the semi-anosmic is indicated in Text-figure 6. In conducting these experiments two fish were found, one in the normal group and one of the fully anosmic group, which showed a strong bias to circle to one side. Evidently there was some asymmetrical influence operating in the sensory mechanism of these individuals. For this reason they were omitted from the main body of the data and are so indicated in Table V and Text-figure 6.

The significance of these data is treated in the discussion.

#### CALCULATION OF RESULTS.

The numerical data obtained in the behavior experiments are capable of being analyzed in a number of respects additional to the simple estimation of the quantitative nature of the repellent or attractive stimulation of the chemical quantities employed.

Considering only the control periods which preceded the experiments, it is desirable to examine them in order to determine if, in spite of experimental precautions, any bias existed in the trough or in the behavior of the fish that might have a bearing on the results of the experiments.

Since it is expected that if there were no bias the fishes should be moving at random, and since in each case here considered there were four specimens in an optically divided trough of two like compartments, it follows that the numerical values of the observations should approximate 50 percent. The actual values observed for each of the four types of fishes used are given in Table VI. None shows any statistically significant departure and the mean of all was found to be 49.88+, that is, the fish were at one end of the tank as often as they were at the other.

Another measure of the presence of a possible bias is to consider the frequency of occurrence of the possible aggregation numbers as compared with the calculated values of the binomial distribution of four items. Since in any observation at one end there can be any of five possible numbers of fish, 0, 1, 2, 3 or 4, it follows from the equation for the binomial formula  $(p+q)^n$ , expanded for each term, that the observed occurrence of 0, 1, 2, 3 and 4 fish together at one end at one time should be 1, 4, 6, 4 and 1 or one chance out of 16 that there will be none, 4 out of 16 that there will be one and so on. The observed values are given in Table VI, from which it is clearly evident that they closely approach the calculated expectancy: 6.25, 25, 37.5, 25, 6.25, being 7, 25, 36, 25, 7. Since the experiments were all carried out using first one and then the other end of the trough, any bias in it would appear if the values obtained at one end be compared with those of the other. Designating one end as "A" and the other as "B," this comparison is also made in Table VI. That there is no significant difference from one end to the other is evident. In this same table there is also given a comparison of all the observations for each form studied. These calculations are shown graphically in Text-figure 7, both with regard to each form of fish and with regard to the trough end. The slight divergencies are clearly indicated. Only in those experiments involving small numbers of observations are any notable divergencies from the binomial values to be seen. Such as are present have a distinct bearing on the problem, but only become clear in the following further analysis.

It was evident during these control periods that each type of fish did not act exactly as every other in respect to their attitudes to their fellows. The above calculations give scant indication of this, however, since whether the fish were acting strictly independently or with some slight degree of unanimity could hardly be expected to show clearly in such a treatment. If the fish were fully tied together, optically or by other means, such behavior would, of course, be evident at once from these fig-



TABLE III. LONG TERM EXPERIMENTS.

Period numbers continue from Tables I and II. This is a continuation of the experiments indicated of those tables and the notation is exactly the same.

EXPERIMENT NUMBER	PERCENTAGE OF FISH IN SUBSTANCE COMPARTMENT											
	9	10	11	12	13	14	15	16	17	18	19	20
La Cueva Chica												
2a (acid)	3 <sup>1</sup>	2	24	22	35	25	20	30	24	26	11	22
2b	30	36	51 <sup>1</sup>	65	51	46	40	44	39	43	51	53
2c	2	0	19 <sup>1</sup>	8	39	34	40	45	52	41	55	62
Pri. means	12	13	31	32	42	35	33	40	38	37	39	46
Sec. means	12		32		39		37		38		42	
La Cueva Chica												
8a (sugar)	51	56	36	44	51	55	41	40	47	40		
Sec. means	54		40		53		40		44			
River Fish												
1a (acid)	44	44	45 <sup>2</sup>	50	54	68						
Sec. means	44		49		61							

## DIFFERENCE IN MINUTES BETWEEN START OF READINGS.

Even numbered readings continuous with preceding odd numbered periods. Odd numbered periods in Tables I and II started about 30 minutes apart, with the following even numbered periods continuous with them.

EXPERIMENT NUMBER	1	3	5	7	9	11	13	15	17	19
2a	—	25	40	25	40	65	55	60	60	60
2b	—	30	40	30	30	75	45	60	60	60
2c	—	25	40	30	30	70	50	60	60	60
Means		27	40	28	33	70	50	60	60	60
8a	—	45	30	30	70	50	60	60	60	
1a	—	25	40	30	30	65	55			

<sup>1</sup> Fish showed distress.

<sup>2</sup> Fish showed distress, with some mucus coagulation.

ures, for instead of a binomial distribution of four items one should obtain a distribution of one item. In other words the figures would indicate the behavior of a school, of four fishes in this case, which under the conditions would give a 1 to 1 distribution value. If the linkage were not as perfect as this case, which might be expected to obtain in a school of herring or mackerel, the values should approach one of the intermediate binomial distributions. Thus with four fish it could be possible to have any of the following frequencies depending on the cohesiveness of the group: 1-1; 1-2-1; 1-3-3-1; 1-4-6-4-1 displaced on the scale of 0 to 4 appropriately. That there is no evidence of a tendency toward the first three possibilities is apparent. The following tabulation of these possibilities and their significance should make this entirely clear.

POSSIBLE FREQUENCIES	SIGNIFICANCE
1-1 1 aggregation.	Four fish stay close together.
1-2-1 2 aggregations.	Groups of 2 and 2 or 3 and 1.
1-3-3-1 3 aggregations.	Groups of 2, 1 and 1.
1-4-6-4-1 4 aggregations.	Four "groups" of 1 fish each.

Attacking the problem in another way, it became apparent in the original paper of

data that in certain cases consecutive observations showed the same number of fishes together for more or less extended time. These sequences of consecutive observations of certain numbers of fish indicated that when the fishes came close together they tended to remain there, which gives some measure of a slight and otherwise scarcely detectable aggregating tendency. This tendency appeared to differ in the various forms. Although this part of the analysis was undertaken because of what appeared to be behavior differences noted during the carrying out of the experiments, the statistical analysis led to a different interpretation of the condition than that based on simple observations.

The mathematical analysis of the occurrence of these sequences of observations of the same number of fish is slightly more complicated than the preceding study of the distribution of the aggregations but is a further rendering of the binomial equation. Since the likelihood of a given event recurring in sequence in such a study is  $p^n$ , it is possible to compare the observed sequences in regard to both their duration in time and number of times they appear in the observations to the probability values obtained for each of the five possible num-



TABLE IV. SHORT TERM PERIODS DURING THE ADMINISTRATION OF SUBSTANCES

Each of these periods are one-quarter the length of those of Tables I and II. They are indicated as lettered parts of periods 5 and 6 of those tables. Each figure represents 25 observations, the means 100.

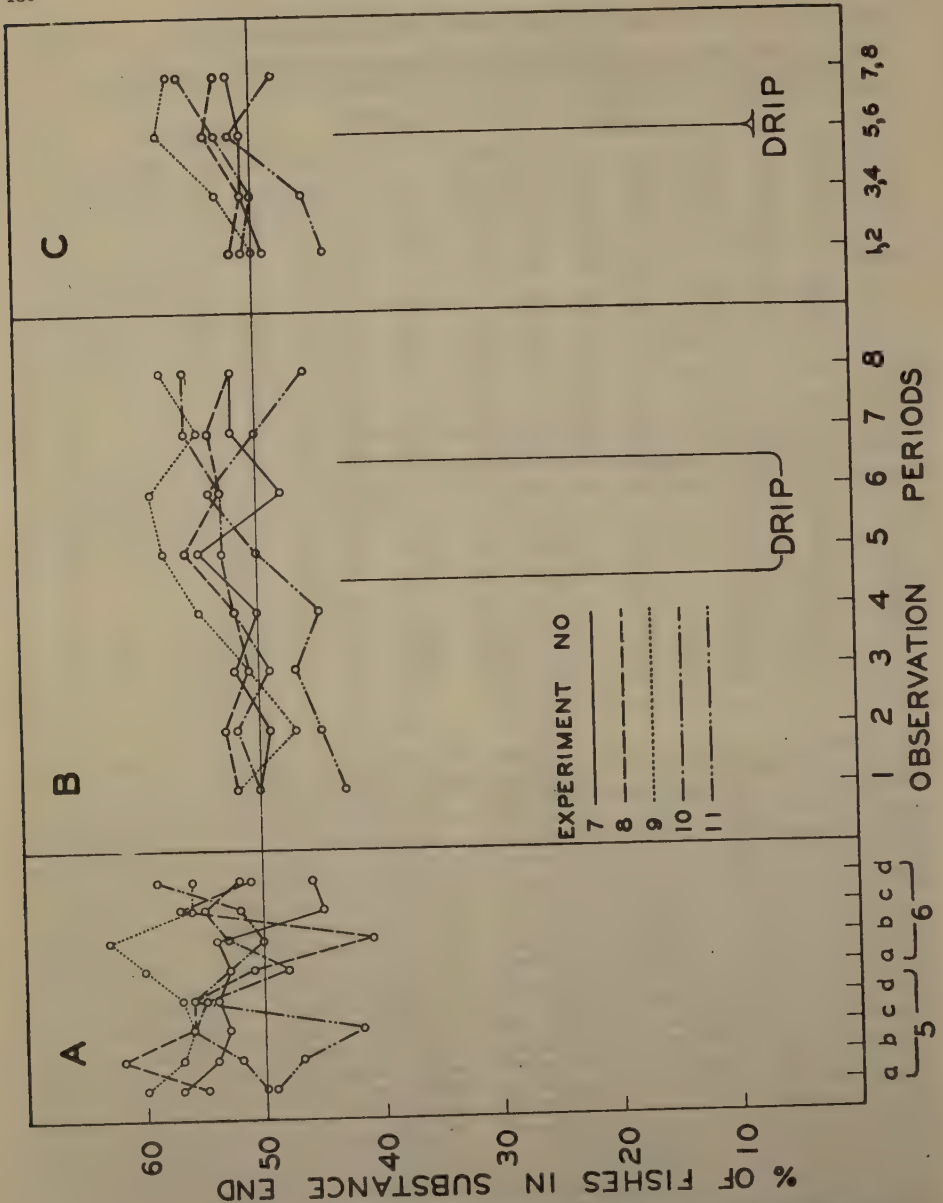
EXP. DRIP PERIODS									EXP. DRIP PERIODS								
No.	5a	5b	5c	5d	6a	6b	6c	6d	No.	5a	5b	5c	5d	6a	6b	6c	6d
1a	56	57	27	17	13	6	3	2	7a	50	56	52	54	56	40	34	48
1b	46	16	19	10	13	3	2	2	7b	68	54	50	67	56	54	44	52
1c	52	50	43	25	11	15	18	7	7c	60	46	52	47	45	49	52	27
1d	47	21	8	3	40	37	11	0	7d	52	59	57	50	55	52	49	56
Means	51	36	24	14	19	16	8	3		57	54	53	54	53	54	45	46
2a	56	37	49	31	17	18	15	7	8a	56	56	54	59	44	49	64	54
2b	48	60	63	39	19	9	5	5	8b	52	61	50	48	54	41	53	54
2c	33	13	2	12	9	11	9	9	8c	59	69	61	59	55	56	54	46
2d	60	64	31	23	4	10	5	9	8d	53	62	58	59	52	49	53	71
Means	49	44	36	26	12	10	8	7		55	62	56	56	51	41	57	51
3a	54	47	70	75	65	73	49	38	9a	75	66	85	77	77	81	80	67
3b	75	42	31	17	19	30	17	8	9b	56	57	46	57	57	57	44	49
3c	50	40	24	35	26	40	37	35	9c	50	51	42	47	46	56	45	50
3d	53	50	45	26	31	31	24	24	9d	58	56	47	59	59	59	56	59
Means	58	45	42	38	38	43	32	42		60	57	56	57	60	63	56	56
4a	54	50	33	27	15	3	6	9	10a	42	53	54	56	56	54	52	58
4b	53	40	16	8	17	5	8	6	10b	43	58	49	51	33	59	56	66
4c	34	32	26	16	16	11	13	11	10c	56	50	62	55	56	55	52	43
4d	76	49	24	12	12	4	4	4	10d	58	49	60	60	48	44	61	50
Means	54	42	25	16	15	6	8	7		50	52	56	55	48	53	55	52
5a	55	64	48	49	42	39	41	52	11a	66	69	60	64	66	63	63	77
5b	62	60	60	53	41	47	41	48	11b	48	28	38	46	34	43	53	64
5c	80	74	68	56	68	55	35	52	11c	50	57	57	75	66	65	64	55
5d	38	36	44	33	27	27	48	33	11d	33	33	35	38	48	30	37	41
Means	59	58	55	48	44	42	41	46		49	47	42	56	53	50	52	59

bers of fish in a group occurring  $n$  times in succession. Divergencies from the calculated values of significant magnitude then appear in two ways: (1) as numbers of times that a sequence of any given length occurs above expectation and (2) actual length of the longer sequences compared with the probability of such a length of sequence occurring at all. The data so obtained for the various experiments of each possible observation in regard to the occurrence of sequences of various lengths is given in Table VII. In this table each experiment is indicated together with the number of times of occurrence of each sequence for every length of sequence observed. In Experiment 1, for example, no fish were at one end of the trough for one observation only 52 times, for a sequence of two observations 17 times and so on, while all four fish were together for one observation only 54 times and for two observations 24 times. Since, obviously, for each set of readings, given in this table, there is another, at the opposite end of the trough, which represents the difference between the total number of fishes, four, minus those indicated in Table VII, it is possible to combine these values and reduce the data to the form given in Table VIII in which

only three sets of figures for each experiment need be handled.

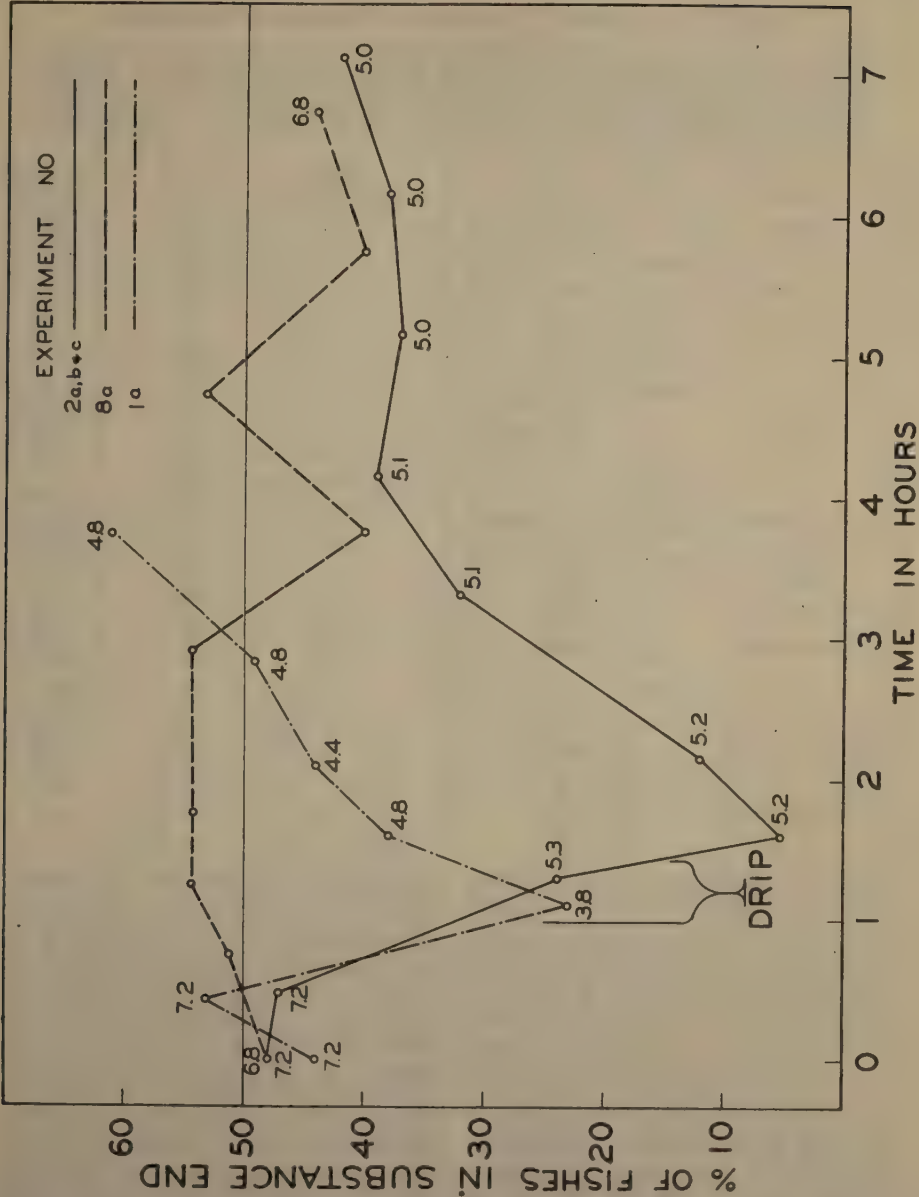
Reducing the calculated probability values to terms in which the first power is equated to unity for each of the different kinds of combinations possible (2 fishes, the mean of 1 and 3 fishes and the mean of 0 and 4 fishes), it is possible to compare these calculated values directly with the observed values by equating the number of single observations (sequences of one) to unity. These figures, based on both observed and calculated values, are given in Table IX, in which the calculated values for each class of data are directly comparable to the observed values for that class. The evident differences show the increase of the observed values both in number of sequences and their length beyond expectancy.

It is evident that while the river and Cueva de los Sabinos fish are closely similar and differ only slightly from binomial expectancy, La Cueva Chica material shows a greater divergence and the anosmics show extreme variance. If the calculated values are subtracted from the observed, then these differences, if positive, show the extent of the observed over the expected and, if negative, the reverse. These values are given in Table X and graphically shown in



TEXT-FIG. 4. Reactions to chemical attractants. Graphic representation of the data of experiments 7 to 11 inclusive, based on Tables II and IV. Exp. 7—Blind river fish, 40% sugar. Exp. 8—La Cueva Chica fish, normal, 20% sugar. Exp. 9—La Cueva Chica fish, normal, 40% sugar. Exp. 10—La Cueva Chica fish, anosmic, 40% sugar. Exp. 11—Cueva de los Sabinos fish, 40% sugar. Graph A: distribution of fishes during periods of the dripping administration of substances. Each point represents 100 observations. Graph B: distribution of fishes before, during and after the dripping administration of substances. Each vertical division four times the size of those in graph A. Each point represents 400 observations. Graph C: distribution of fishes as in graph B but with all consecutive observations represented as a single point. Each point represents 800 observations.





TEXT-FIG. 5. Long term experiments with repellents and attractants. Graphic representation of the data of experiments 1a, 2a, b and c and 8a, based on Tables I, II and III. Exp. 2a, b and c—La Cueva Chica fish, normal, acetic acid 10%. Exp. 8a—La Cueva Chica fish, normal, sugar 20%. Exp. 1a—Blind river fish, acetic acid 10%. Each point of experiments 1 and 8 represents 200 observations and of 2 each point represents 600 observations. The verticals which have been referred to a time scale are equivalent to those used in graph C of text-figures 3 and 4. The figures at each point indicate the pH value at that time at the end of the trough remote from the administration of the substance.

TABLE V. INFLUENCE OF NOSTRILS ON SWIMMING DIRECTIONS.

Each sub-experiment covers an interval of five minutes. Clockwise turns in the case of the left anosmic are with the intact nostril to the inside of the turn. The normals and left anosmics found food in the first interval and were then discontinued. The full anosmics did not find food through three sub-experiments. In no case was the sugar actually found because of its going into solution. For this reason the full anosmics were omitted from this test.

EXP. NO.	FISH	STIMULUS	TURNS CLOCKWISE	TOTAL TURNS	PER CENT CLOCKWISE
12a	Normal	None	9	18.5	48.6
12b			12	23.5	51.1
	Means			21	50
13a	Left anosmic	None	15	15	100
13b			10.5	13.5	85.2
	Means			14	93
14a	Full anosmic	None	10.5	21.5	53.5
14b			9	21.5	41.8
	Means			21	48
15a			8	16	50
15b	Normal	Cane sugar	10	19.5	51.3
15c			6.5	14.5	44.8
	Means			17	49
16a			6	10.5	57.2
16b	Left anosmic	Cane sugar	11.5	12.5	92
16c			8	11.5	69.6
	Means			11	73
17	Normal	Food	6.5	14.5	44.8
18	Left anosmic	Food	25	29.5	84.9
19a			7.5	20	37.5
19b	Full anosmic	Food	14.5	24	60.4
19c			10.5	21.5	48.8
	Means			22	49
20a			11	17	64.6
20b	Biased normal	None	14.5	17.5	82.8
20c			11	13	84.6
	Means			16	77
21	Biased full anosmic	None	25	30	83.4

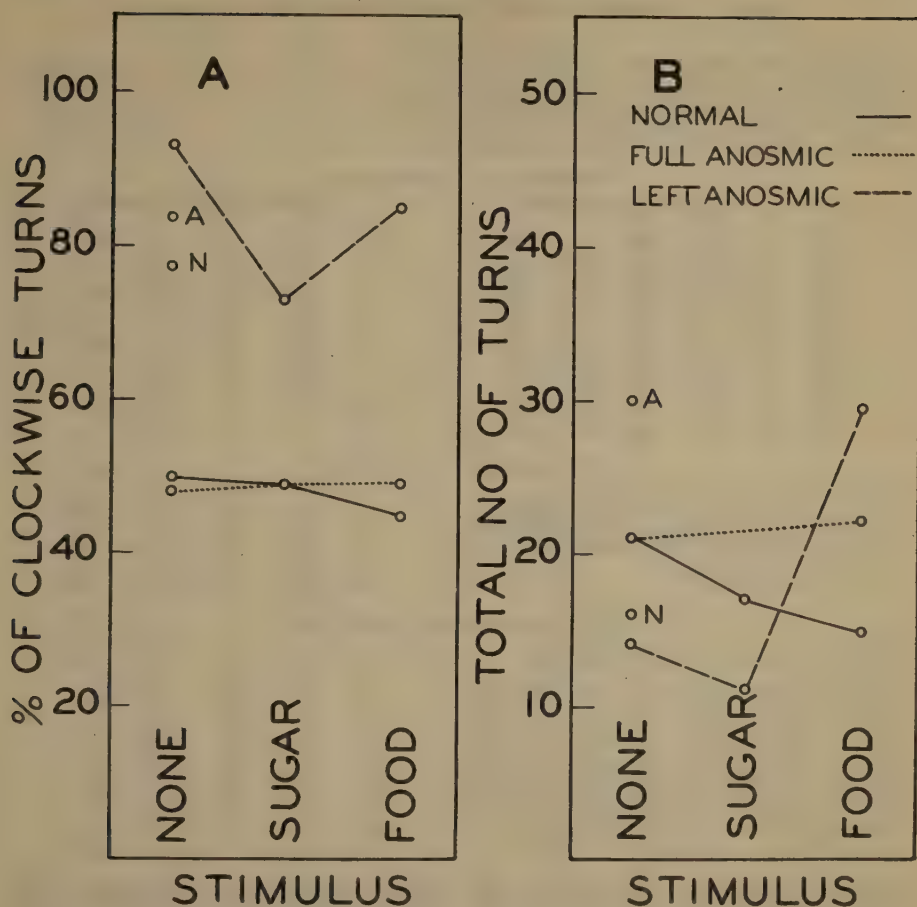
Text-figure 8. Here in effect the calculated values are represented by the horizontal "O." A full discourse on these implications is reserved for the discussion, but the following may be noted here. This type of analysis clearly shows that all exceeded the calculated values but that while the river and Cueva de los Sabinos fish showed the least departure from expectancy, the anosmics showed the most and were approached by La Cueva Chica normals. Observations at the time of the experiments seemed to indicate that the first two actually gave more heed to their fellows than did the others. This gave an appearance of an aggregating tendency but this analysis, on the other hand, could be taken to indicate that the anosmics grouped more than the others. Evidently what appeared to the eye in observing the fishes was that the river and Cueva de los Sabinos fish detected each other with greater certainty or at a greater distance from each other but, nevertheless acted with greater individual independence.

Thus what was actually seen was evidently these fishes turning toward each other or attempting to avoid collisions. This feature has considerable bearing on certain of the aspects of the effects of repellents and attractants which is subsequently developed.

This method of analysis is not useful for the periods involving application of substances or in the post-application periods because of the increasing complication arising from the presence of a gradient in time and space of the chemical involved to which can be assigned no accurate numerical value. In other words, since the fish were pushed or pulled toward one end of the trough, the significant separation of the effects of the chemicals from those of aggregating tendencies becomes impossible by this method. The simpler treatment of employing arithmetical means, furthermore, suffices for the later periods.

No significant correlations could be established between the various experiments or their parts that bore any relationship to





TEXT-FIG. 6. Circling movements of normal, anosmic and unilaterally anosmic fishes and their relative number per unit of time. Graph A: percent of turns toward the intact nostril of semi-anosmics as compared with similar turns of normal and fully anosmic fish in reference to various stimuli. Graph B: total number of turns per five minute interval of the three types of fish in reference to various stimuli. Based on the data of Table V. See text for full explanation.

the slight variations in the pH values reached or the number of cc. used as recorded in Tables I and II. Evidently these differences were all below any value that might be expected to show measurable differences in the fishes' behavior by the methods here employed.

An analysis of the frequency distributions of the data of Tables I and II and the original figures on which they are based showed no significant differences in dispersion coefficients. Consequently calculations of the standard deviations are omitted.

#### DISCUSSION.

It is notable that the extent of reactions to repellents as compared with attractants is much more marked in the former, as may be seen by a comparison of Text-figures 3 and 4. This would be expected since a repellent would be operative under practically any circumstance while the extent of influence of such an attractant as might be used would depend on many factors, considering what slight influences will stop feeding reactions in fishes. Such items as extent of hunger, fright, sexual or other conditions

TABLE VI. DISTRIBUTION OF FISHES DURING CONTROL PERIODS.

		ARITHMETIC MEANS									
Blinded river fish (Exp. 1 & 7; periods 1 to 4).....		50 50									
La Cueva Chica normals (Exp. 2, 5, 8 & 9; periods 1 to 4).....		51.08 +									
La Cueva Chica anosmics (Exp. 3 & 10; periods 1 to 4).....		48.07 +									
Cueva de los Sabinos (Exp. 4 & 11; periods 1 to 4).....		48.06 +									
All (Exp. 1, 2, 3, 4, 5, 7, 8, 9, 10 & 11; periods 1 to 4).....		49.88 +									

DISTRIBUTION OF AGGREGATIONS													
EXP.	NO. OF	AGGREGATIONS OBSERVED						AGGREGATIONS IN PERCENT					
NO.	OBS.	0	1	2	3	4	5	0	1	2	3	4	5
1	1600	101	386	589	412	112	—	6	24	37	26	7	—
2	1600	156	389	496	418	141	—	10	24	31	26	9	—
3	1400	112	384	364	276	64	—	8	27	40	20	5	—
4	1600	121	396	510	432	141	—	7	25	32	27	9	—
5	1600	96	368	605	410	121	—	6	23	38	26	7	—
6	200	54	27	24	14	28	53	27	13	12	7	14	27
7	1600	85	407	617	387	104	—	5	25	39	24	7	—
8	1400	71	333	520	386	90	—	5	24	37	28	6	—
9	1584	88	359	619	393	125	—	5	23	39	25	8	—
10	3200	746	1667	787	—	—	—	23	52	25	—	—	—
11	1600	191	440	543	345	81	—	12	27	34	22	5	—

AGGREGATIONS GROUPED BY FORMS					
Blinded river fish (3200 observations)	6	25	37	25	7
La Cueva Chica normals (6184 observations)	7	23	36	26	8
La Cueva Chica anosmics (1400 observations)	8	27	40	20	5
Cueva de los Sabinos (3200 observations)	10	26	33	24	7
All (13984 observations)	7	25	36	25	7
Calculated expectancy	6.25	25	37.5	25	6.25

AGGREGATIONS GROUPED BY TROUGH END					
Blinded river fish ("A" end; 1600 obs.)	6	27	39	22	6
Blinded river fish ("B" end; 1600 obs.)	5	23	37	28	7
La Cueva Chica normals ("A" end; 3000 obs.)	5	23	35	29	8
La Cueva Chica normals ("B" end; 3184 obs.)	8	24	38	23	7
La Cueva Chica anosmics ("A" end; 800 obs.)	10	32	35	18	5
La Cueva Chica anosmics ("B" end; 600 obs.)	3	21	50	22	4
La Cueva Chica anosmics ("A" end; 1600 obs.)	24	52	24	—	—
La Cueva Chica anosmics ("B" end; 1600 obs.)	22	52	26	—	—
Cueva de los Sabinos ("A" end; 1600 obs.)	9	25	33	26	7
Cueva de los Sabinos ("B" end; 1600 obs.)	11	27	33	22	7

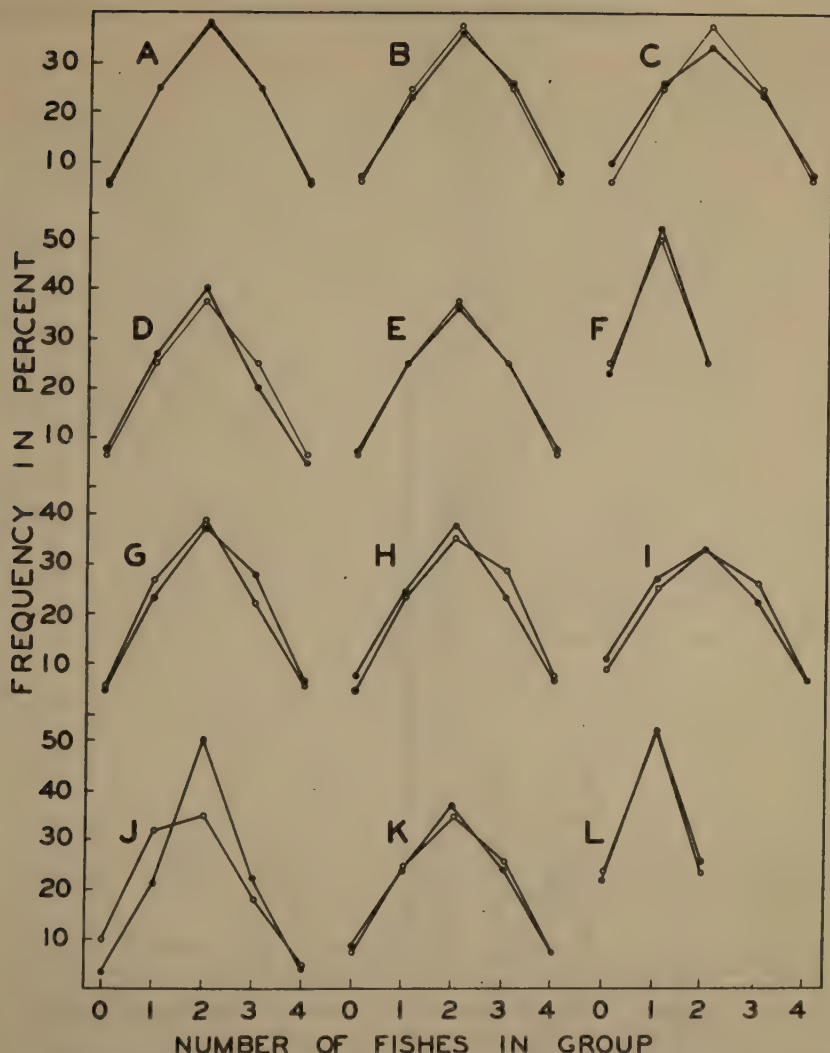
all would be expected to have more or less inhibitory effects from time to time. In other words a repelling influence would be expected to have a more consistently definite effect than a potentially equally attractive influence.

Since integumentary response in fishes is lodged both in the taste buds and in the nerve endings of the "common chemical sense," the latter of which is evidently only stimulated by sour, saline and alkaline solutions, Parker (1922), these skin sensations require some discussion. It was not feasible in our work to attempt experimentally to separate these two sensory systems, nor would it have served any important purpose in the present study. This condition may well represent, however, a further reason for the difference in the magnitude of the reactions to a sour and a sweet substance. In this connection it should be borne in mind that Strieck (1924 and 1925) has shown that *Phoxinus* can recognize sub-

stances ordinarily tasted, by means of the nasal organs alone, since in water any chemical is freely presented to both receptors because both are bathed in a common solution.

Considering first the retreat of these fishes before an increasing gradient of acetic acid, it is evident that there is no clear statistical difference between the normal fishes, in respect to nostrils and taste buds, of any of the three types under study. Three kinds of irritation may enter into this avoiding reaction; taste bud influences, nasal pit influences and general skin irritation, "the common chemical sense." The differences in taste bud number and the nature of the nasal pits, between the three types, would hardly be expected to show a graphic difference by this manner of treatment. However, it must be admitted, graphs B and C of Text-figure 3, which are based on the largest body of data and least subject to incidental influ-





**TEXT-FIG. 7.** Observed frequency distribution of fishes in all possible groupings compared with the calculated binomial distribution of the same number of items. Based on the data of Table VI. Graphs A to F: distribution of fishes by forms (dark circles and heavy lines) compared with binomial distribution (open circles and light lines). A: blinded river fish, experiments 1 and 7. 3,200 observations. B: La Cueva Chica fish, experiments 2, 5, 8 and 9. 6,184 observations. C: Cueva de los Sabinos fish, experiments 4 and 11. 3,200 observations. D: anosmic fish, experiment 3. 1,400 observations. E: all above experiments, 1, 2, 3, 4, 5, 7, 8, 9 and 11. 13,984 observations. F: anosmic fish, experiment 10. 3,200 observations. Graphs G to L: distribution of fishes by forms in reference to end of trough employed. Open circles represent the "A" end and dark circles the "B" end. G: blinded river fish, experiments 1 and 7. "A," 1,600 observations. "B," 1,600 observations. H: La Cueva Chica fish, experiments 2, 5, 8 and 9. "A," 3,000 observations. "B," 3,184 observations. I: Cueva de los Sabinos fish, experiments 4 and 11. "A," 1,600 observations. "B," 1,600 observations. J: anosmic fish, experiment 3. "A," 800 observations. "B," 800 observations. K: all above experiments, 1, 2, 3, 4, 5, 7, 8, 9 and 11. "A," 7,000 observations. "B," 6,984 observations. L: anosmic fish, experiment 10. "A," 1,600 observations. "B," 1,600 observations.







TABLE IX. OBSERVED SEQUENCES AND CALCULATED BINOMIAL DISTRIBUTION.

CALCULATED BINOMIAL DISTRIBUTION (FIRST POWER = UNITY)

POWER	FOUR ITEMS			TWO ITEMS	
	2	1 - 3	0 - 4	1	0 - 2
1	1.00000	1.00000	1.00000	1.00000	1.00000
2	.37500	.25000	.06250	.50000	.25000
3	.14062	.06250	.00392	.25000	.06250
4	.05273	.01562	.00024	.12500	.01562
5	.01978	.00392	.00002	.06250	.00392
6	.00742	.00098	.00000	.03125	.00098
7	.00278	.00024		.01562	.00024
8	.00104	.00006		.00781	.00006
9	.00039	.00002		.00392	.00002
10	.00015	.00000		.00195	.00000
11	.00005			.00098	
12	.00000			.00048	
13				.00024	
14				.00012	
15				.00006	
16				.00003	
17				.00001	
18				.00000	

OBSERVED DISTRIBUTION OF FISHES. (NO. OF SINGLE OCCURRENCES = UNITY)

SEQUENCE OF FISHES	MEAN			MEAN			MEAN	
	2	1-3	0-4	2	1-3	0-4	1	0-2
	River	(1 & 7)		Chica	(2, 5, 8 & 9)		Anosmics (10)	
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	.422	.394	.352	.442	.432	.333	.542	.389
3	.160	.143	.033	.218	.153	.120	.268	.093
4	.074	.052	.014	.096	.058	.048	.164	.024
5	.026	.013	.005	.044	.018	.033	.095	.006
6	.017	.003		.010	.020	.006	.047	.002
7	.002			.012	.008	.012	.017	.002
8	—			.008	.008	—	.008	
9	—			.005	.002	.003	.006	
10	—				.002	.003		
11	.005				.002	—		
12	.002				—	.006		
13					.001			
24					.001			
	Sabinos (4 & 11)			Anosmics (3)				
1	1.000	1.000	1.000	1.000	1.000	1.000		
2	.385	.352	.274	.476	.685	.625		
3	.131	.160	.097	.208	.272	.187		
4	.054	.049	.051	.128	.211	.187		
5	.028	.027	.021	.128	.078	.031		
6	.010	.020	.013	.059	.070	.062		
7	.010	.007	.004	.030	.026	—		
8	—	.002		.040	.035	.031		
9	.002	.002		.010	.035	—		
10				.020	—	—		
11				.010	.009	.031		
12				—	.009	—		
13				.010	—	—		
14				.010	—	—		
16				—	.009	—		
26				.010	—	—		
31				.010	—	—		

ences, show in the final period that the three types have retreated from the irritant in the order of their increasing number of taste buds, or in order of the nasal capsule changes, if such be considered as positive. Herrick (1903) showed that the delicacy of the sense of taste was directly propor-

tional to the number of taste buds in a given area. He worked on a variety of teleosts, including *Ameiurus*.

The anosmic specimens clearly did not avoid the irritant to nearly as great an extent as did the normals. It would thus appear that such avoiding reactions are



TABLE X. DIFFERENCE BETWEEN OBSERVED SEQUENCES AND BINOMIAL DISTRIBUTION.

These values obtained by subtracting the binomial distribution values of Table IX from the observed distribution of fishes from the same table.

SEQUENCE OF FISHES	RIV.	CHICA (2)	SAB.	ANOS.	RIV.	CHICA (1 - 3)	SAB.	ANOS.
1	.000	.000	.000	.000	.000	.000	.000	.000
2	.047	.067	.010	.101	.144	.182	.102	.435
3	.019	.077	— .010	.047	.081	.091	.098	.210
4	.021	.043	.001	.075	.036	.042	.033	.195
5	.006	.024	.008	.108	.009	.014	.023	.074
6	.010	.003	.003	.052	.002	.019	.019	.069
7	— .001	.009	.007	.027		.008	.007	.026
8	—	.007	—	.039		.008	.002	.035
9	—	.005	.002	.010		.002	.002	.035
10	—			.020		.002		—
11	.005			.010		.002		.009
12	.002			—				.009
13				.010		.001		—
14				.010		—		—
16				—				.009
24				—		.001		—
26				.010				—
31				.010				—

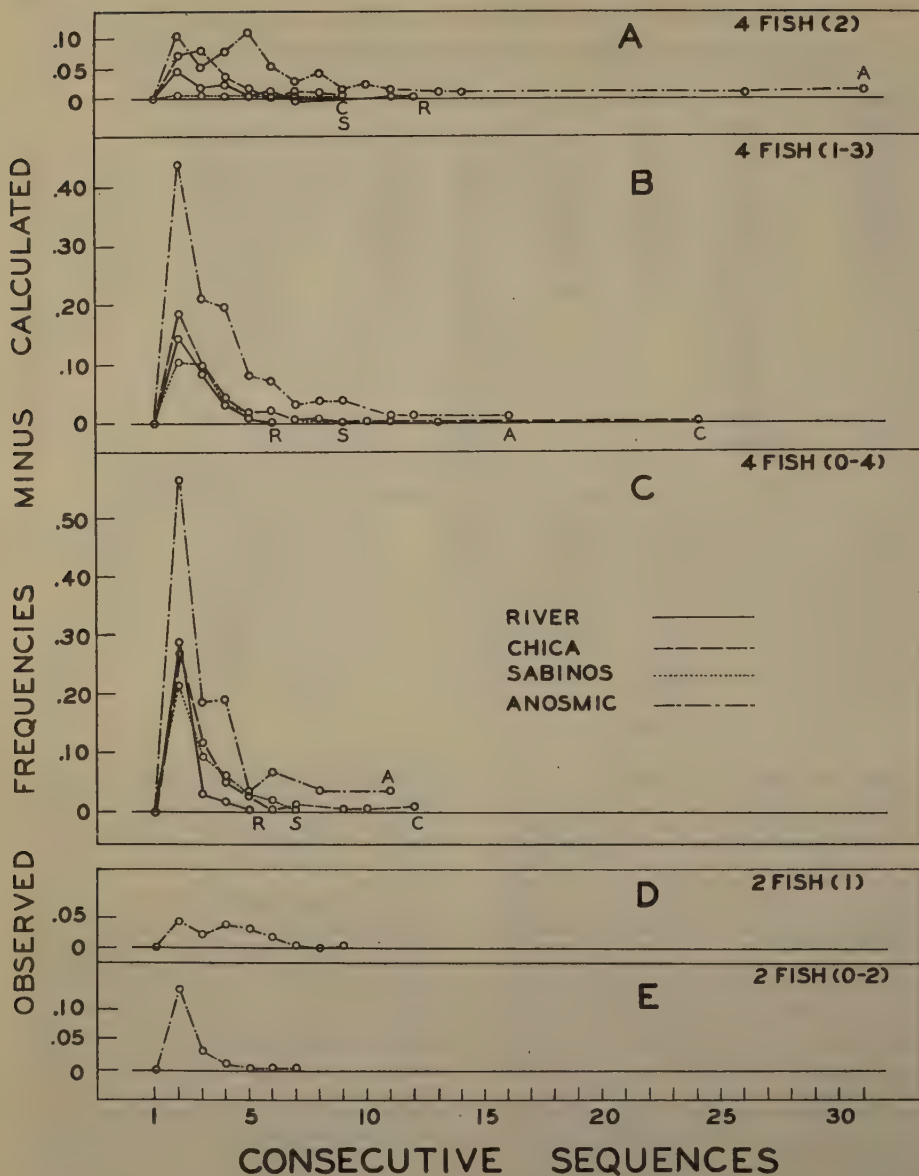
ANOSMICS			
	(0 - 4)	(1)	(0 - 2)
1	.000	.000	.000
2	.289	.270	.211
3	.029	.116	.093
4	.014	.048	.051
5	.005	.033	.021
6		.006	.013
7		.012	.004
8		—	
9		.003	
10		.003	
11		—	
12		.006	

largely mediated by way of the olfactory apparatus. This could lead to the conclusion that the difference shown in graph B of Text-figure 3 (26 per cent., considering the range of from 0 to 50 as 100 per cent.) is a measure of the extent of the directive utility of the taste buds and the common chemical sense alone. The exact method of operation could be considered as occurring in two possible fashions. One would be that such chemical irritation is largely nasal and the other would be that the nature of the sensory perception is unimportant, but that part of it which happens to be nasal gave a much greater discriminatory ability with the consequence that the fish were able to react with more markedly appropriate action. One would hardly expect the range-finding ability of the diffuse taste bud and common chemical sense systems to be as accurate in locating the focus of a diffusing irritant as the discrete bilaterally paired nasal organs, even if the sensation in them were of a minute order. Expressed another way, retreat from an irritant is possible on taste and skin irritation alone but not with nearly as great accuracy as with the advantage of the more certainly directive nasal organs. It has already been indicated

by Parker (1922 and earlier) that the common chemical sense endings are stimulated by acid but these workers did not undertake to show how directive this response might be without the orientating nasal mechanism.

About all that can be said concerning the repellent characteristics of ammonium carbonate is that it is evidently much less of an item to be avoided than acetic acid in the same percentage solution. From these experiments it would be pointless to consider the relative ionization of the two substances in aquarium water with its mixtures of organic materials. Much further elaborate work involving the chemistry of the whole matter would have to be done in order to attempt to equate comparative effects of cation and anions, which is certainly only a small part of the entire matter, and in any case beyond our present province.

It is to be noted that there was some evidence of the fishes being at first slightly attracted to both the acid and the base. This is indicated in Text-figure 3, graph C and Table IV. In the case of the ammonium carbonate it is very marked and was noted during the progress of this experiment, for



TEXT-FIG. 8. Comparison of observed consecutive sequences of groups of fishes with distribution of probabilities of  $p^n$ . Based on the data of Tables VII, VIII, IX and X. Graphs A, B and C represent the data from experiments 1 and 7, river fish; 2, 5, 8 and 9, La Cueva Chica fish; 4 and 11, Cueva de los Sabinos fish; 3, anosmic fish. Graphs D and E represent the data from experiment 10, anosmic fish. See tables and text for full explanation.



in the early part of the dripping period the fish clearly gathered about the inflow at some point of evident equal concentration and were then pushed outward as this line of equi-potential moved forward. Actually it was first thought that this substance would have to be listed as an attractant and it is possible that it should be considered such in certain low concentrations. Normal eyed goldfish were used as a check on this item, the details of which are given in Table I, experiment 6, but not shown in Text-figure 3. Two additional periods, 9 and 10, not given in Table I, had values of 27 and 37 respectively. It is thus apparent from these data that the figures for the goldfish, from equality of distribution, rose sharply in the dripping period to very high values, 56 and 76, and then fell immediately to low values, 36, 32, 27 and 37, giving means of paired periods of 34 and 32. This is evidently merely an exaggeration of the conditions found in the characins. This may mean that goldfish are simply more reactive to ammonium carbonate as compared with the characins or that, since they were using visual cues and are a strongly aggregating species, any response would give the appearance of greater reaction because of the group tending to follow any individual that moves out from the aggregation. During this experiment one each of the following species were also present in the trough, but not counted: *Danio malibaricus* (Jerdon) *Lebistes reticulatus* (Peters), *Xiphophorus hellerii* Heckel, *Betta splendens* Regan and *Tilapia macrocephala* (Bleeker). These, while not aggregating with the *Carassius auratus* (Linnaeus), clearly showed similar behavior. It thus seems evident that certain low concentrations of ammonium exert an attractive influence at first and subsequently act as a repellent. Although somewhat masked in the graphs it was evident during the observations that the acetic acid acted in a similar fashion for a very brief period. This would seem to indicate that these and probably most fishes move toward practically any weak stimulus at first and then retreat from it if found noxious. This is evidently true of sound and mechanical shock as mentioned by Breder (1942).

The use of cane sugar as an attractant was established after various food juices, such as solutions of prepared dried food, horse meat extract and tubifex worm extract had been used and were found to exert no greater influence, as has been already noted. The others were abandoned for these tests because of technical difficulties in standardizing their quantities. Sheldon (1909) showed that while an elasmobranch, *Mustelus*, was generally sensitive to acids and alkalies in very dilute solution and less so to salts and bitter substances, they did not react to sugars, a feature certainly not

common to teleosts in general. For example, Trudel (1929) shows clearly that *Phoxinus laevis* Agassiz reacts to various sugars and indicates that the taste thresholds are, for the most part, not vastly different than in man. Krinner (1934) reviews the reactions of teleosts to NaCl. As noted in another connection, Strieck (1924 and 1925) has shown the interrelation of the sense of olfaction and taste in fishes. The characins under study, as has been indicated in the various tests, are evidently about as reactive to cane sugar as they are to meat juices and other possible food substances. This is evidently not so clearly the case, on the basis of the work of others, with different species of fishes. Kuroda (1932) has shown, by measuring changes in the respiratory behavior of *Ophiocephalus argus* Cantor, that sweet, sour, bitter and saline substances each induce a distinguishably different type of respiratory modification. This work was done on fishes held in a fixed position so arranged that the movement of the operculum traced a line on a kymograph drum. The exact significance of the variations of these movements in response to solutions of various gustatory substances is not clear, but his work suggests the possibility that further study by such a method might prove of value in present connections. Due to the small size of these characins, a more delicate arrangement than that used by Kuroda was constructed which, while mechanically satisfactory, could not be used with these fish. It was found that the slightest touch on the operculum would cause the fish to change its respiratory behavior in such a fashion as to reduce the movement of the operculum, in reference to the contact, to such minute proportions that mechanical amplification was completely impracticable. Due to the large variety of ways in which these fish can manipulate their opercular and branchiostegal apparatus, it was found impossible to so truss them up as to force them to make use of opercular movements against even the most delicate contact.

In addition to the interference of incidental influences inherent in the use of an attractant by such methods already mentioned, it must be borne in mind that fishes generally show notably rapid learning or conditioning. These specimens would go through their normal feeding reactions at first and when, on not finding anything palpable, would give up trying to find food. If, on the other hand, they had been presented with food at this time, they would have fed and then retreated because of a satisfied appetite, which indicates one of the difficulties in such an approach. The complications and irregularities in these experiments are evident in Text-figure 4, graphs A and B. It is not until the material is consolidated as in graph C that a fair estimate

of the effects may be obtained, which differs clearly from the relatively clear cut results obtained when an irritant was used. Here it is evident that the river fish showed a gradually increasing interest in the sugary taste, but of slight order. The normal Cueva Chica fishes showed a much stronger positive reaction to the same concentration, 40 per cent., while not such a strong one to 20 per cent. The Cueva de los Sabinos material showed the sharpest reaction to the sugar drip but quickly lost interest. The anosmic fishes showed more of a reaction than did the river fish and finally nearly reached the level of the normals in the last period. This is in accord with the general observations on the anosmics, to the effect that they take a materially longer time to locate and complete their feeding. Whether olfaction is involved here or not would be hard to establish in other than the sense discussed in connection with the repellent effect of acetic acid on the anosmics.

The longer term effects of both acid and sugar are indicated in Text-figure 5. As the period of observations were separated by varying lengths of time, this chart has had a time scale incorporated in the abscissa and pH values are given at the various observation periods. In the two lines concerned with acid it will be noted that there was a tendency to return to the normal figure. La Cueva Chica material, however, never did reach the equality value, even after both sides of the trough had reached the same pH value and there was no longer any directive differential. This may be due to a memory of the location of the source of the irritant, as it has been shown that these normally blind fishes have a strong memory of the location of obstacles, as was indicated by Breder and Gresser (1941b). The blinded river fish, on the other hand, nearly reached equality before the pH values had become the same at both ends and as soon as it reached that value spent more time in what was initially the repellent end. Since these fish, up to the time of blinding, were dependent to a large extent on visual cues, it is possible that they did not retain such a strong memory of the location of the irritant. At least they did not, during this same period, have the adroitness in avoiding obstacles that the blind fish showed. This final strong bias to the acid end of the trough may have, in fact, been largely a matter of complete confusion.

The long term analysis of reactions to sugar shows the loss of interest in what was at first an attractant and perhaps a final irritation to it. Naturally there was no pH change involved and it is assumed that there was complete dispersion of the sugar solution before the end of the experiments, on the basis of the behavior of the acid and the

mixing caused by the constantly moving fishes.

Another approach to this analysis is that of considering the circling motions which these fish regularly make in regard to a unilateral anosmic condition. Table V and Text-figure 6 give the data of these studies. It is evident from the latter, graph A, that the normal and completely anosmic fish turn substantially as many times right as they do left in the presence of sugar, food or with no stimulus. The unilateral anosmics, on the other hand, show a strong tendency to turn toward the side of the intact nostril. In graph B it will be seen that the actual number of turns of both anosmics and normals were about the same in the column of no stimuli. The latter make less turns for the sugar and still less for the food. As the number of turns possible was related to the length of duration of the experiment, this falling off is due to their stopping or swimming straight ahead while actually feeding. It will be noted that the anosmics did not slow their pace. The semi-anosmics, on the other hand, slowed their pace for the sugar while they sought the unfindable stimulus and greatly increased it for actual palpable food. In the latter case they were impelled to make turns to one side only, which slowed their actual food finding. The process of locating a food particle served to increase their gyrations, thus accounting for their large number of circles. For some reason, not altogether clear, they usually turned toward the side of the missing nostril when engaged in chewing their food. Possibly it was a momentary negative response to additional food odors while they were actually engaged in mousing a particle. Adrian and Ludwig (1938), for example, found that in the oscillographic recording of nerve impulses from the nasal capsule of *Ameiurus*, "A period of stimulation which has produced a considerable discharge is usually followed by a period of inactivity lasting 5-20 sec. or more. During this period the organ is insensitive to a second stimulus and the normal resting activity is greatly reduced."

It appears that what have been here classified as "repellents" and "attractants" are actually not truly separable on a qualitative basis for it must be recalled that the attractants also, in long term experiments, eventually exerted a repelling influence, while the repellents exerted an initial attraction. Stated in general terms the basic reaction would seem to be to move or turn toward any stimulus and then retire from it, irrespective of whether it be noxious or beneficial. The extent of the approach and retreat and the change in reaction in point of time would then be conditioned by (1) the specific attitude and physiological con-

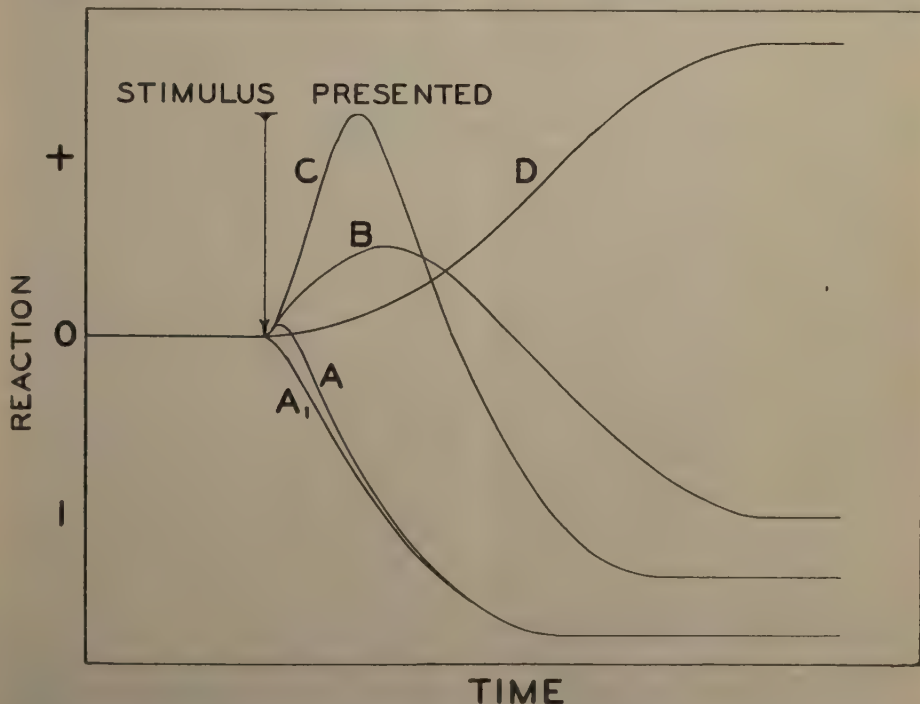


dition of the fish, (2) the violence of the stimulus and, (3) the chemical and physical nature of the stimulus. Considered in this manner, then, the various and superficially different behavior items in fishes in such a regard may be discussed in the following terms.

If the stimulus be sufficiently violent and sudden, as in the case of a heavy jolt, the positive reaction may be reduced to nearly or actually zero, an immediate retreat being all that is detectable. Anything short of such an item should then show an early turning toward or moving toward the stimulus, such as found in these experiments, followed by a retreat, the time and violence of which would be conditioned by the three characteristics listed above. In any case it is well known that the violence of both mechanical and chemical stimuli determines whether the reaction will be positive or negative. Parker and Van Heusen (1917) showed it very clearly for electrical stimuli as well. This would follow even if the stimulus were food which was found and eaten.

Probably only in the case of multiple factors would a permanent shift be obtained, which in its effects would practically amount to those involved in initiating a migration. The causes of movement in or out of a cave, as discussed by Breder and Gresser (1941b), would be of such a nature.

Text-figure 9 represents graphically an idealized concept of this general proposition. Much more work, not appropriate to the present paper, must be done to test thoroughly the validity of this view. Obviously any line, in addition to those hypothetical ones shown, could be drawn on such a chart. Those indicated, as based on present data and on general considerations, would seem to be some which do approximate reality. For example it seems that there is little likelihood of a line rising from the zero mark that would simply return to it and not show a movement to the negative side. After a reaction, obviously the lines would not remain horizontal, as shown, for an indefinite period. They would sooner or later return to the zero level (by



TEXT-FIG. 9. Hypothetical diagram of the quantitative nature of reactions to various stimuli. A, A<sub>1</sub>: stimuli in which a positive reaction is practically or completely absent. B: mild stimuli showing a slight attraction and subsequent repulsion. C: strong stimuli showing a marked attraction and a subsequent strong repulsion. D: a mild but permanent strong attraction. See text for full explanation.

a process of integration?) when a balance was once more established due to neutralization of the stimulus, its failure to give continuing directive effect, accommodation of the fishes or other matters, except in cases of the permanent establishment of a bias, where, as noted above, the permanent shift of a population would be involved.

This concept could be extended to include such cases where a fish retreats rapidly before a sudden splash and then later returns to the site of the disturbance, which apparently happens when the shock of the fright has fallen to a level where it is no longer a repellent.

In the development of an increased number of surface taste buds in this series of fishes the evolutionary changes involved are a little difficult to visualize. If one takes the selectionist view point and ascribes a selective advantage to the fish possessing a few more taste buds one would have to assume a restricted food supply. Such a condition does not obtain at present in La Cueva Chica where the bottom is floored with food substances, Breder (1942). Cut off from such a situation, dependent on a population of bats, a condition of limited food could be fairly considered but it is hard to see how a few more taste buds could be of much practical significance to the owner. Due to the continual random swimming of the species, each fish in time would cover all the area and the advantage of the better equipped fish would have to be on the basis that it covered a little wider "ribbon" or simply missed less food particles, a matter to be significant certainly calling for a fair increase in number of taste buds before selection could be expected to operate. Since the development of taste buds is evidently dependent on nerves developing first, Olmsted (1920), May (1925) and Torrey (1936), selection would apparently thus have to operate on cutaneous branches of gustatory nerves. Since the development of a new single branch would certainly call for the development of more than one taste bud, presumably at least several, it may be that a superficially slight neurological change would call forth a selectively significant increase in the number of taste buds.

Before the increase in the number of taste buds had been determined, Breder (1943) wrote, "... it may well be that these two blind populations are independent developments from the same basic stock and differ only in the accidental assortment of genotypes that gave rise to them." The intermediate nature of the taste bud tracts and the nasal structures, as well as other physical details not concerned with the present study would certainly seem to suggest that the Cueva Chica stock does in truth represent a stage in the evolution of the Cueva de los Sabinos population rather

than that the two forms represent independent but parallel developments. In this same paper it is stated, "The chemical senses are apparently normally developed in these fish, but do not seem to be hypertrophied. They show positive and negative reactions similar to those of various surface fishes," and "Histological examination shows no significant structural modifications in the fish from either cave." Also, "Thus, as far as present evidence goes, at least, we have a case of an intrusion into a 'simpler' environment with a loss of sensory refinements but with no evident substitute structural developments." The subsequent work, reported herewith, clearly changes the aspect of some of these statements for while it is still true that no histological or gross morphological changes have appeared, nor is there any evidence of a hypertrophy of the chemical senses, there are small morphological changes in the nasal capsule and changes in the number of external taste buds, at least the latter coming under the head of a positive change.

Considering the analysis of the distribution of the fishes during the control periods, some curious features appear. Regarding the distribution of the four types of fishes studied, it is clear from the tables and graphs that the river fish and those from Cueva de los Sabinos resembled each other in the behavior so expressed more closely than did the others. Also these two departed least from the expected distribution and their values are scarcely over the limits of statistical significance. The Cueva Chica fishes showed a greater departure from the expected distribution and when deprived of the sense of smell developed a still larger difference. This fact, coupled with the conditions noted during the observation periods in which the river and Cueva de los Sabinos fish appeared to be more cognizant of each other than did the rest, would seem to be difficult to reconcile. Thus if the data are tabulated by order of the tendency to aggregate, entirely contrary evidence is obtained between the visual impressions and the statistical treatment, as indicated below.

FISH	ORDER OF MAGNITUDE OF AGGREGATING TENDENCY	
	Statistical analysis	Visual impressions
Blinded river fish	1	3
Cueva de los Sabinos	1	3
La Cueva Chica	2	2
Anosmic	3	1

Disregarding for the present that the above two series of orders of magnitude are inverse, certain properties are common to each. The river fish and Cueva de los Sabinos fish are alike in both columns, the anosmics are at the other extreme and La Cueva Chica fishes are intermediate between them and the first two. Considering first only this feature of the tabulation, it should,



no doubt, be expected that the specimens deprived of the sense of smell would be set apart from the rest, as they are. Since La Cueva Chica material has been shown to be intermediate between the river fish and those from Cueva de los Sabinos in many ways, it is difficult to see why in the present respect the two end terms of the series are alike. Since the river fish, up to the time of blinding, showed strong aggregating tendencies based on acute vision, it may be that they employed some undetected mechanism used at night or in muddy water for the recognition of each other. This mechanism may be no different than that possessed by La Cueva Chica fishes, but the river fish may have simply paid more attention to each other through the same mechanism, because of past conditioning. The fish from Cueva de los Sabinos, on this basis, may have this, or some other mechanism, developing along with their taste bud and nasal changes so that they show similar behavior.

However one may wish to interpret these data it would seem clear that the sense of smell is involved in some manner, as is evidenced by the great difference in behavior of the anomic fishes. Frisch (1938, 1941a and b) and Göz (1941) have shown that *Phoxinus laevis*, *Ameiurus nebulosus* (LeSueur) and *Alburnus bipunctatus* (Bloch) secrete a substance which can be detected by their fellows. Positive reactions are shown toward such materials (skin extracts) but retreat is shown to similar extracts of *Esox*, *Perca* and *Salmo*. In some cases individual recognition appears possible. Juices from an injured *Phoxinus* cause alarm in a school. These effects are apparently entirely olfactory. Hüttel (1941) discusses the chemistry involved. Bowen (1931) could not assign a role to olfaction in the aggregations of *Ameiurus melas* (Rafinesque). The present study is evidently the first to show by statistical methods that blind fish, both normals and operates, actually do demonstrate aggregating tendencies based on non-visual cues, and that measured by the standards of optically integrated groups it is a very feeble and loose structure. A considerable list of species and references to the visual nature of fish schools and aggregations is given by Breder and Nigrelli (1938). See also Shlaifer (1942). It is entirely possible that in any or all of these species a similar mechanism exists, for none of the work on the subject has been carried into such statistical analysis as the present. In all, the schools are simply broken up with a coming of darkness, while actually the disrupted schools certainly could not superficially show any less of an aggregation than did some of the present specimens. See, for example, Parr (1927) or Breder and Nigrelli (1935). In addition to the sense of smell being evi-

dently involved, the role of the lateral line, taste buds or other items is still to be explored.

It is of interest to note in this connection that Hoagland (1933) by oscillographic recordings of nerve impulses found that tactile stimuli to the barbels of *Ameiurus* gave large responses but that those from chemicals, such as acetic acid up to 20 per cent. solutions, 10 per cent. solution of NaCl and saturated sugar solutions gave scarcely detectable responses. He found that these tactile responses could be elicited by slight movements of the water. This condition may account for the relatively slight responses obtained with our chemicals and may indicate a truly large significance in regard to water movements. Breder and Gresser (1941a and b) have already established the large significance of rheotropic effects in these fishes which, except in the eyed river fish, cannot be optical in the sense shown by Lyon (1905, 1909a and b) and Loeb (1918) but are presumably akin to the tactile reactions such as those discussed by Jordan (1917) and Maxwell (1921a and b).

The puzzling inversion between the statistical analysis and the visual impressions is partly explained at an earlier place. It should be recalled that these measurements were made in a trough of two equal divisions and any fish was noted as either in or out of the compartment being checked. Thus the fishes might be close together or relatively far apart and still be counted in the one compartment, or, on the other hand relatively close together but counted as one in and one out. Actually this did occur in the case of the Cueva de los Sabinos fishes, for in several cases they tended to spend considerable time near the middle of the trough, evidently paying some attention to each other. Contrariwise it was not uncommon for the fish to spend a considerable amount of time running the circuit of the periphery of the trough. The fact that the river fish and the Cueva de los Sabinos fish did relatively little of this, spending more time in the central portion of the trough, certainly contributed to the impression that they were more cognizant of each other. This difference in behavior in itself very likely could be taken as a further measure of their tendency to awareness of each other, on the basis of the work of Breder and Nigrelli (1938) who showed that a solitary goldfish spent most of its time around the periphery of an aquarium and that a conveniently sized group spent most of their time at its more central parts. Obviously this change in behavior would not be reflected in the statistical analysis in a simple fashion. Thus the two inverse orders of magnitude between statistical measure and visual impressions may be reconciled on the

following basis. Because the river fish and the Cueva de los Sabinos specimens spent more time in the middle of the trough they appeared to be paying more attention to each other than to the periphery of the trough, and, evidently they were, but statistically they frequently split their group over the dividing line, thus failing to give the long and numerous sequences of a single group found in the others. The Cueva Chica material, spending so much time circumnavigating the trough, with attention to each other subsidiary, when once close together tended to stay that way to a greater degree. When these fish were made anosmic their basic behavior continued the same but not impinged upon by olfactory stimuli and guided, chemically, only by taste, their behavior appeared more deliberate and they seemed to show an exaggeration of the conditions found in them before becoming anosmic. The statistical values taken alone could be interpreted as meaning that the loss of the sense of smell made it possible for these fishes to detect each other more readily, certainly a ridiculous conclusion. Evidently, on the other hand, when they did locate each other, they tended to remain in company for longer periods than any of the others, either because of tasting each other, because of water movements or other unknown effects, or because of tending to move at a more uniform rate, not being impinged upon by the sharply bilateral nasal sensations.

That the nasal organs of fishes are comparable with those of terrestrial vertebrates and are used primarily as distance receptors for chemical entities as compared with taste buds and the common chemical sense, has long been established by a number of students. Reference to the bibliographies of the following will give a full history of the subject: Parker (1910, 1911, 1914 and 1922), Parker and Sheldon (1913), Sheldon (1911), and Olmsted (1918) together with many of the other references here mentioned in other connections. These workers concerned themselves chiefly with *Mustelus*, *Ameiurus* and *Fundulus*.

Experiment 6, on five goldfish, only referred to in connection with the discussion of the ammonium carbonate experiments, shows a marked difference from the behavior of any of the characin experiments in regard to present items of distribution. Both the distribution of the aggregations, Table VI, and the distribution of the sequences, Table VII, are inverted in respect to the characins. In both, the end terms are high and the central terms low. These fish had their full vision and seemed to be bothered by both the shallowness of the water and the fact that there was no cover whatever. The five of them were much more apt to be huddled together at one end or the

other as is indicated by the value of 27 per cent. for five and 27 per cent. for zero descending to 12 per cent. for 2 fish and 7 per cent. for 3 fish, the condition showing the greatest amount of dispersion. This clearly is not binomial distribution but rather an approximation of its reciprocal. It was for reasons such as this that it was necessary to blind the river fish, because of their extreme nervousness, in this case amounting to their jumping out of the trough. This is one reason that the relatively placid goldfish was used. It is interesting to note that the evidently highly exciting optical stimuli are apparently entirely responsible for the nervous condition of the river fish, for as remarked earlier, on being blinded, their general behavior becomes that of the cave fish, which is one of relative stability.

It has been shown in the earlier papers of this series that the evolution displayed by these fishes in retreating into a cave environment has involved losses, as follows: pigmentation, vision and ear structure. The present study shows that along with those losses there has been a concomitant gain in taste, while olfaction may eventually fall as either a gain or a loss. Although there is structural modification of the nasal capsule, behavior changes are evidently not great enough to be clearly measurable by the methods of the present studies on sensory response.

The blinded river fish, quite evidently little disturbed by their new condition, displayed the darkest color phase of which the species is apparently capable. The range of color change in these fishes is not great, but the behavior in this respect was typical of fishes in general on the loss of vision, see for example Odiorne (1937) who discusses similar color changes in blinded *Ameiurus*. The taking on of the wandering movements of the cave fish was not exactly expected, since other fishes such as trouts and sunfishes tend more to "sulk" in a corner under similar circumstances. This could mean that in spite of the large optical control evident in these river fish, they may be more active at night than one would ordinarily suppose. If this be true it would be another factor encouraging original cave entry. In any case Breder and Gresser (1941b) have indicated that these fishes show no hesitancy to enter dark places on slight encouragement. Evidently they are constitutionally so disposed.

#### SUMMARY.

1. There is a notable increase in the number of taste buds on the head and body from the normal-eyed river fish to the blind fish of La Cueva Chica and a still further increase to the more advanced fish from Cueva de los Sabinos.



2. In the same series and in like order the nasal capsules become more shallow and open but show a reduction in the number of sensory lamellae and consequently in the extent of sensitive epithelium. This is accompanied by an increase in the development of the directive nasal flaps.

3. The first positive development in the evolution of this series is shown to be in the taste mechanism and perhaps also in the olfactory mechanism, other sensory apparatus thus far studied having all shown reductive changes.

4. Avoidance reactions to chemical repellents is substantially the same for all three types, with a possible slight increase in sensitivity from the river fish to the more advanced cave forms.

5. Attention to chemical attractants is similar but not as well marked or differentiated.

6. Anosmic specimens avoid repellents not nearly as well as those with nostrils and take longer to locate attractants, evidently taste alone offering inferior orientation.

7. Fatigue, confusion or distress, any or all, neutralize and may tend to invert the negative or positive reactions in one to three hours.

8. Semi-anosmic fish show a strong bias to turn toward the side of the functional nostril, with stimuli either present or absent, indicating the orientation control to be largely nasal in these fishes.

9. The primary nature of reactions to either noxious or beneficial stimuli is suggested as being basically the same, differing only in a quantitative sense.

10. Non-visual influences concerned with the limited aggregating tendency of these fishes evidently involves the nasal capsule, in part at least, for anosmic specimens showed behavior different from the others.

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**EXPLANATION OF THE PLATES.****PLATE I.**

Comparison of nares in Mexican characins.

UPPER: from Rio Tapaon, 45 mm. in standard length.

MIDDLE: from La Cueva Chica, 52 mm. in standard length.

LOWER: from Cueva de los Sabinos, 51 mm. in standard length.

**PLATE II.**

Comparison of nasal capsules in Mexican characins.

UPPER: from Rio Tapaon, 34 mm. in standard length.

MIDDLE: from La Cueva Chica, 29 mm. in standard length.

LOWER: from Cueva de los Sabinos, 32 mm. in standard length.

Each section represents a transverse cut through the posterior nostril in a homologous region, median side to to the right. Compare with Text-figure 1 for location of these sections and their interpretation. Magnification 57.5  $\times$ .

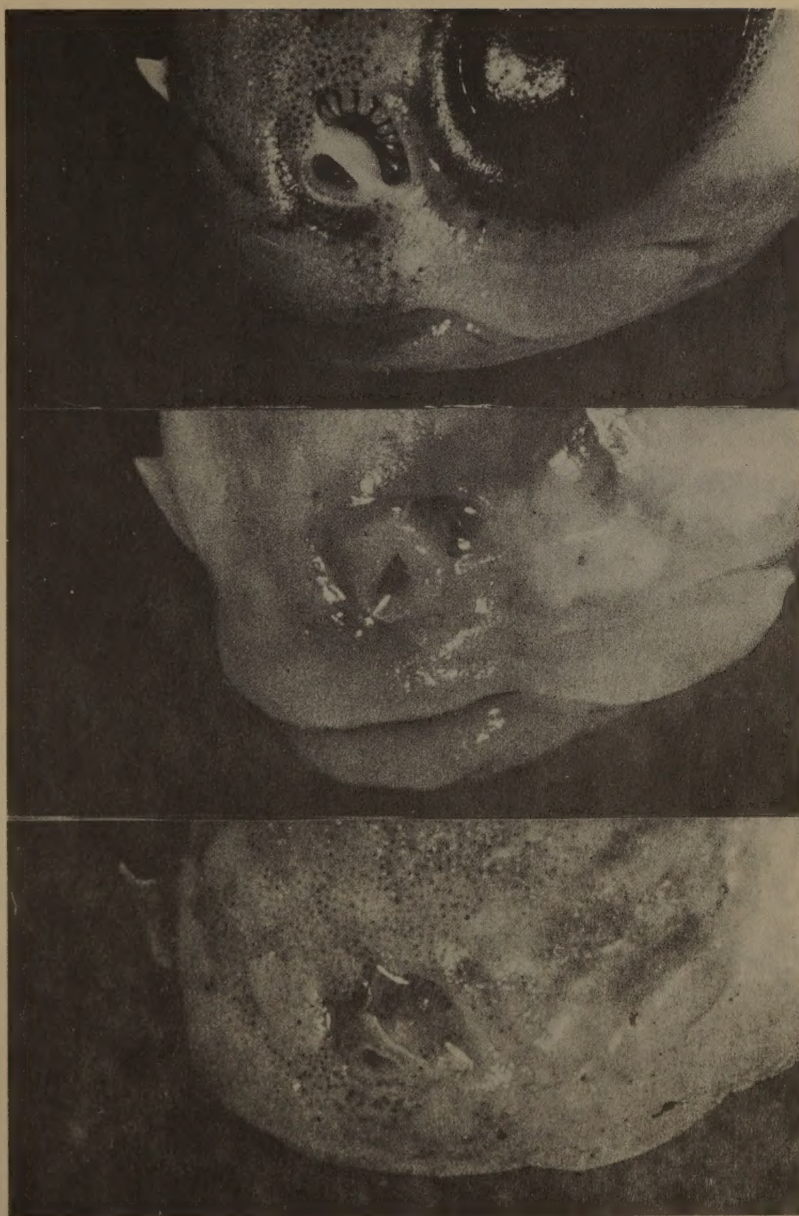
**PLATE III.**

Comparison of taste buds from various regions of the body from a La Cueva Chica specimen of 29 mm. in standard length.

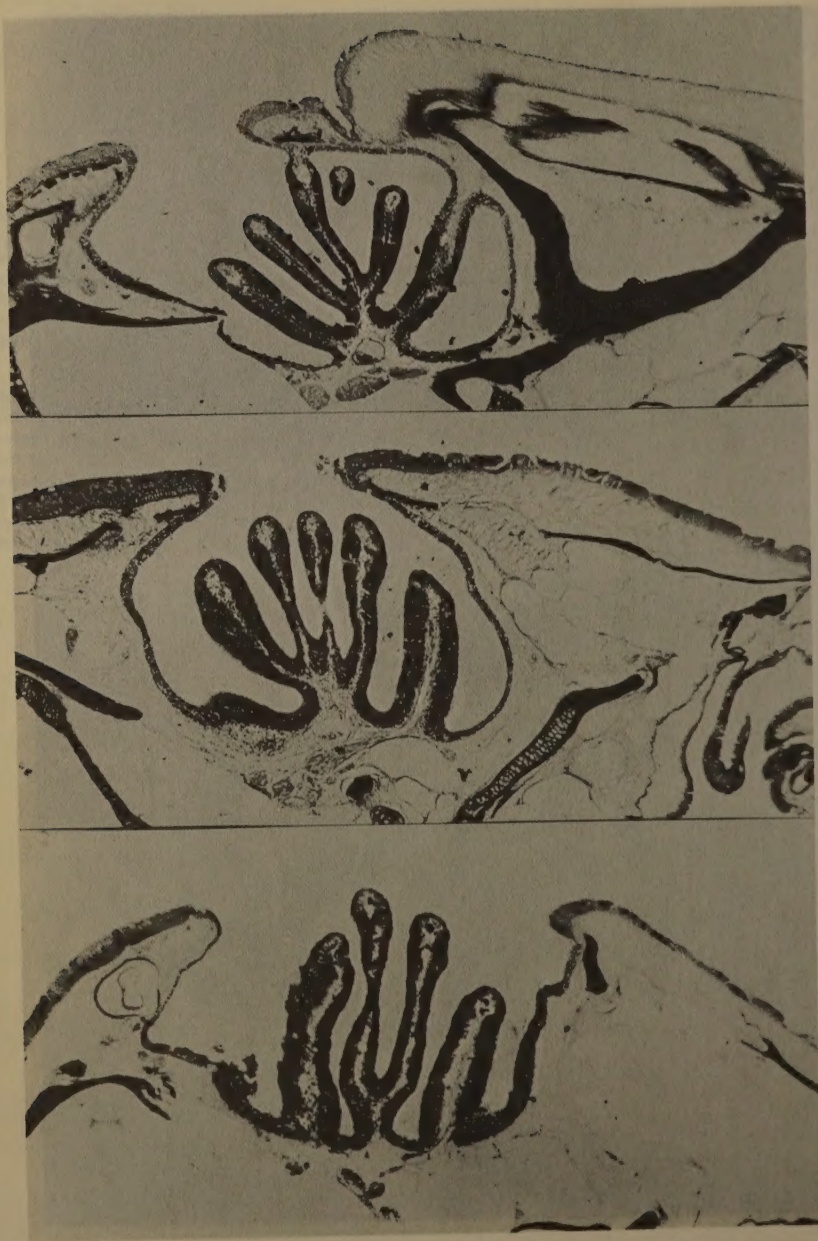
UPPER: from interior of buccal cavity.

MIDDLE: from exterior of head.

LOWER: from lateral portion of body. The dark layer at the lower part of this photograph represents a scale. See text for full explanation and Text-figure 2 for the distribution of these organs. Magnification 695.5  $\times$ .

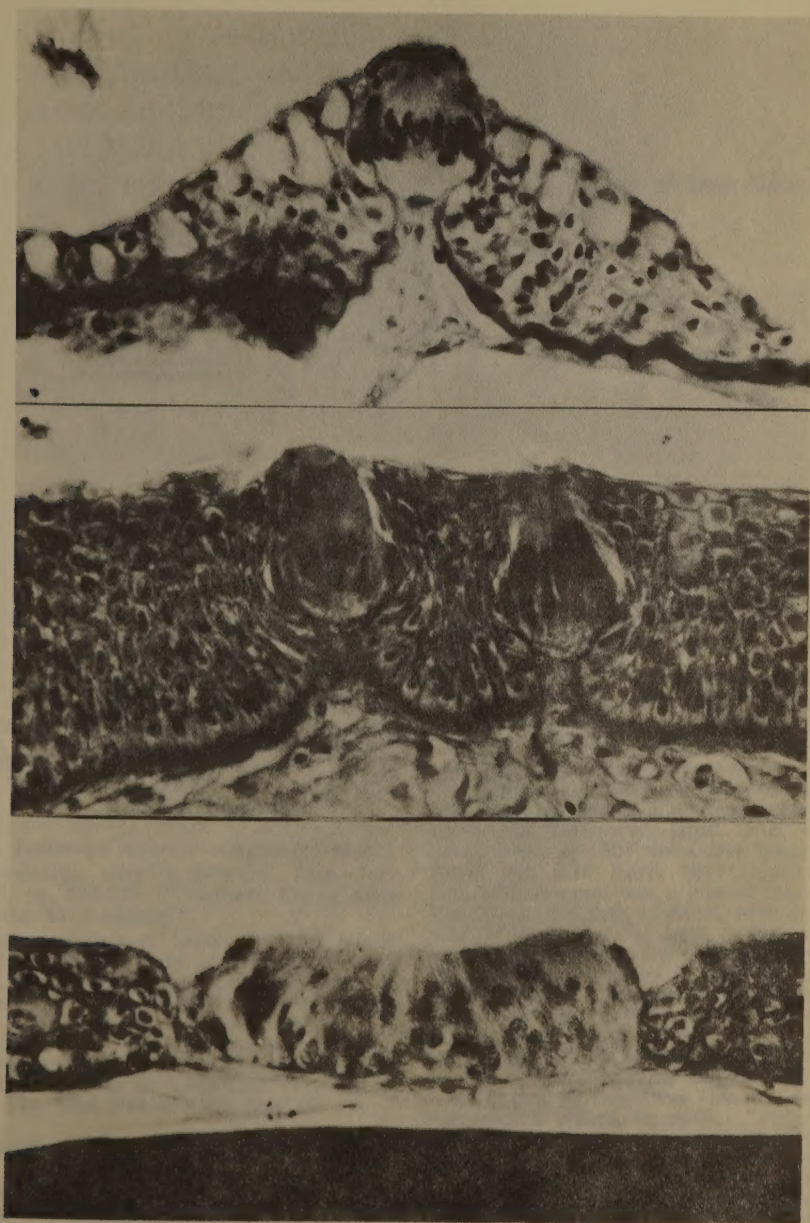


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